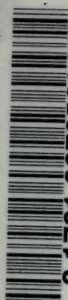


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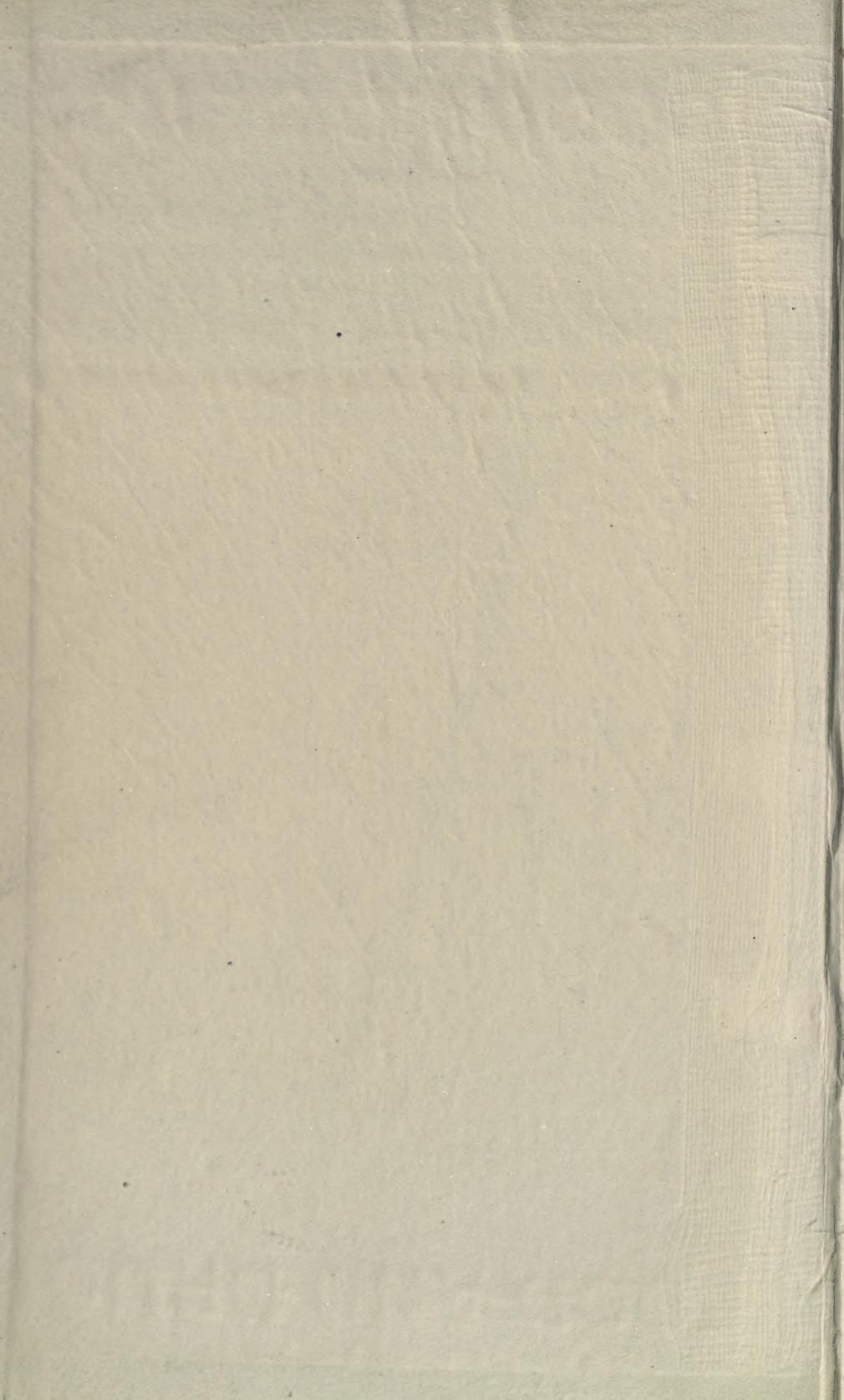
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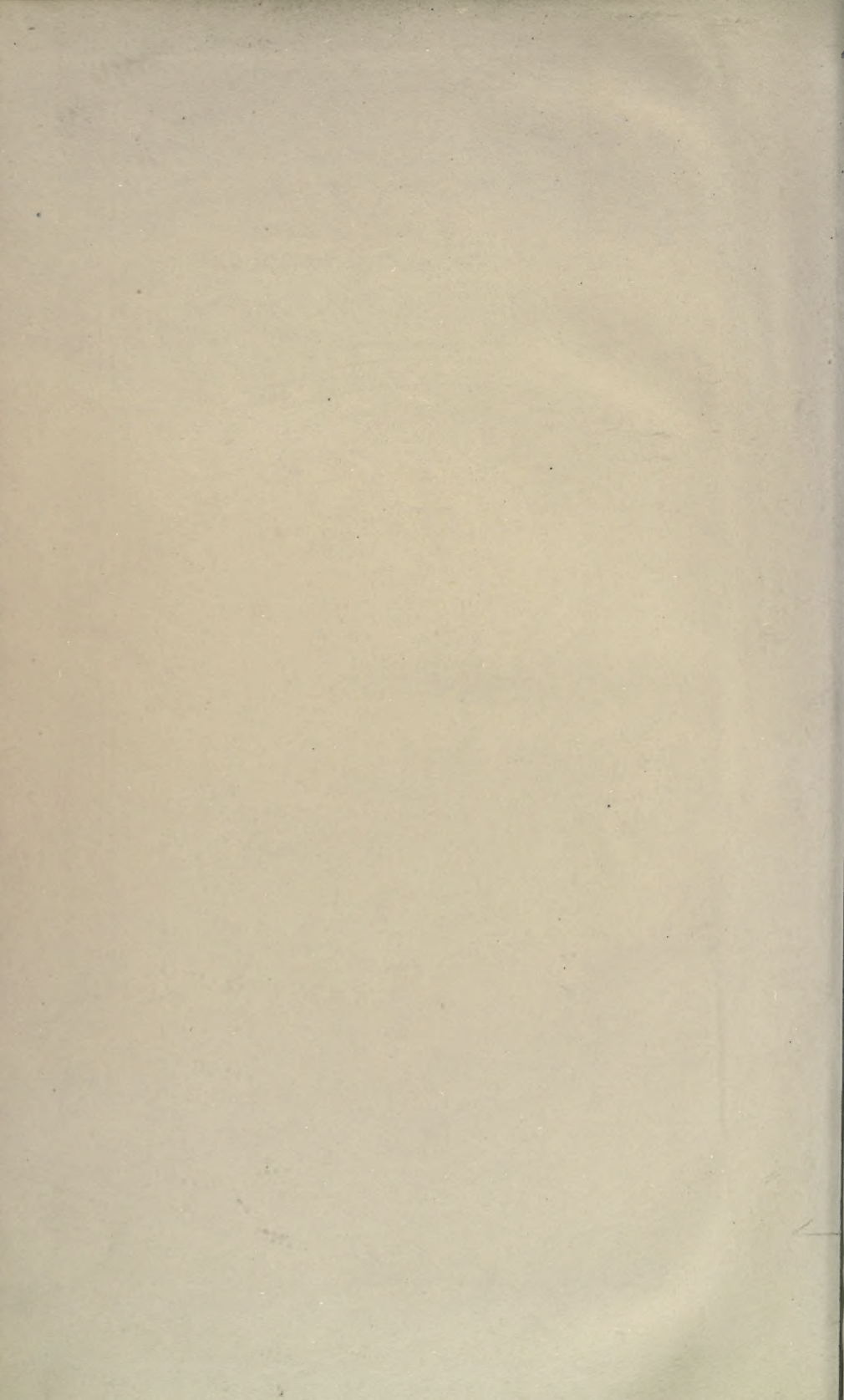
THE ANALYSIS OF FOOD AND DRUGS
(CHEMICAL AND MICROSCOPICAL)

ERNEST J. PARRY, B.Sc.(LOND) F.I.C., F.C.S.



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FOOD AND DRUGS

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FOOD AND DRUGS

BY

ERNEST J.^{ohn} PARRY, B.Sc. (LOND), F.I.C., F.C.S.,

MEMBER OF THE SOCIETY OF PUBLIC ANALYSTS, ETC.,
BARRISTER-AT-LAW, OF GRAY'S INN

VOLUME I

THE ANALYSIS OF FOOD AND DRUGS
(CHEMICAL AND MICROSCOPICAL)

WITH FIFTY-NINE ILLUSTRATIONS

LONDON

SCOTT, GREENWOOD & SON

"THE OIL AND COLOUR TRADES JOURNAL" OFFICES

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STUDY AND WORK IN THE
UNIVERSITY OF TORONTO

(1911)

PREFACE.

IN the preparation of this work the aim of the Author has been to deal with the question of food and drugs from both the chemical and the legal points of view, in a manner that will be of assistance to those entrusted with the administration of the Sale of Food and Drugs Act, primarily, and also to those engaged in the examination of food and drugs for other purposes.

It is hoped that the application of chemical knowledge to the legal aspect of the question, as set out in the second volume may prove of service to counsel and solicitors engaged in cases under the Acts.

I have to acknowledge the kindness of Professor Greenish in writing the chapter on microscopic analysis; and to thank Messrs. Cecil Cribb, Peter MacEwan, E. A. Pinchin, A. Searl, and J. C. Umney, for much assistance in the reading of proofs; and Mr. H. Droop Richmond for several valued suggestions. My acknowledgments are also due to the Editor of the "Pharmaceutical Journal" for permission to use numerous illustrations for which he holds the copyright.

I am also much indebted to Mr. R. J. Preston, LL.D. (Lond.), for reading the proofs of Volume II.

ERNEST J. PARRY.

THANET HOUSE,
56A GREAT DOVER STREET,
LONDON, S.E., *July*, 1911.

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PART I.
CHAPTER I.
TEA, COCOA AND COFFEE.

TEA.

TEA, as used as a beverage, is the dried and prepared leaf of various species of *Thea*, a shrub belonging to the genus *Camellia*. *Thea sinensis* is the principal species from which the tea leaf is derived, but several other species are also employed, of which the most well recognized are *Thea viridis*, *T. Bohea*, and *T. Assamica*. The differences between the many kinds of tea known in commerce do not, however, depend on any important botanical distinctions, but on methods of preparation, age of the plant, time of gathering of the leaf, etc. The finest teas are derived from the young leaves of young shrubs, the old leaves and leaves of old plants being of inferior quality. Black tea has undergone a certain amount of fermentation before it is dried, whilst green tea is the result of rapid drying of the leaves before fermentation has set in.

The only questions in reference to tea that the analyst is called upon to decide are in regard to its purity or otherwise, and occasionally in reference to the amount of caffeine (theine) and tannic acid present. The quality of tea has practically no relation to its analysis, and the expert tea taster is the recognized authority in reference to the quality and value of tea.

The adulteration of tea, especially when it was a very expensive product, was most gross and very common, and the importance of the matter was recognized as far back as 1724 when the Adulteration of Tea and Coffee Act was passed (11 Geo. 1, c. 30). Sect. 5 of this Act imposed a penalty of £100, with forfeiture of the tea, for any adulteration whatsoever. In 1730 an Adulteration of Tea Act was passed (4 Geo. 2, c. 14), which goes further and inflicts a penalty of £10 per pound weight of the tea adulterated, and makes penal the use of exhausted tea leaves. In 1776 a further Act was passed (17 Geo. 3, c. 29). The former Acts only touched dealers in tea, whilst this Act imposed a penalty on any person who adulterated the tea, or had it in possession. In 1875, the Sale of Food and Drugs Act of that year, sect. 30, provided for the examination of all tea imported into this country by the Customs authorities, since which date adulteration has been far less common.

Sect. 31 of the same Act defines exhausted tea, as "any tea which

has been deprived of its proper quality, strength or virtue by steeping, infusion, decoction, or other means”.

Apart from the analysis of tea as an article of food, tea is frequently tested for its percentage of caffeine. Caffeine of commerce (which substance is identical with theine) is manufactured from tea, and tea of inferior quality is used for this purpose. It is allowed into this country duty free if denatured by the addition of such substances as render it impossible for use as a beverage. In these cases the value of the tea is obviously in direct proportion to its caffeine content.

The Composition of Tea.—According to Eder (“Dingl. Poly. Journal,” 131, 445, 526) the average composition of tea is as follows:—

Soluble in water.			Insoluble in water.		
Moisture	10	per cent	Chlorophyll	1.8-2.2	per cent
Tannin	10	“	Wax	0.2	“
Gallic and oxalic acids	0.2	“	Resin	3.0	“
Quercetin	0.1	“	Colouring matter	1.8	“
Boheic acid	2	“	Extractive matter	16.0	“
Caffeine	0.6	“	Cellulose	20	“
Essential oil	12	“	Albuminous matter	12.7	“
Albuminous matter	3 to 4	“	Mineral matter	4	“
Carbohydrates	1.7	“			
Mineral matter					

Of these, caffeine is the principal constituent of tea. It is an alkaloidal compound of the uric acid series (a trimethyl-xanthine) and of the formula $C_8H_{10}N_4O_2$. It was originally described, when separated from tea, under the name of theine, but was later shown to be identical with caffeine, the alkaloid of coffee, under which name it is now generally known. It crystallizes with one molecule of water. Its melting-point is about 233° . In large doses caffeine exerts a poisonous effect, but in moderate doses finds useful employment in medicine.

The essential oil which has been stated by Eder to exist to the extent of 0.6 per cent was possibly obtained from a scented tea. At all events, Van Rombugh only obtained 0.006 per cent from a genuine tea. Schimmel & Co. have examined two samples which they obtained from fermented leaves and suggest that the oil may be a resultant of the fermentation process. These two oils had specific gravities 0.866 and 0.856 respectively, and were only faintly optically active. The oil contains methyl salicylate and an alcoholic body of the formula $C_6H_{12}O$. Acetone and methyl alcohol were found in the distillation water.

Quercetin is a compound, possibly of the formula $C_{15}H_{10}O_7$, which is found to a minute extent in tea. It is of no importance from the analytical point of view.

A glucoside quercitrin, $C_{21}H_{32}O_{12}$, is also said to be present in minute amount. On hydrolysis this splits up into rhamnose and the above-mentioned quercetin.

The remaining constituents of tea do not require discussion.

The following figures represent ten samples of tea—five of ordinary Ceylon tea sold in shops at from 1s. 6d. to 2s. 6d. per lb., and five of ordinary China tea of the values 2s. 6d. to 3s. 6d. per lb., which have been analysed by the author:—

	Ceylon Tea.					China Tea.				
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Moisture	7.9	8.5	10	11.1	7.7	8.2	9.8	7.6	8.8	9
Caffeine	3.3	2.9	3.7	2.6	2.9	2.5	2.9	3.6	2.9	3.8
Tannin	12.5	14.1	16	13.8	17.5	9.4	11	10.2	9	10
Ash	4.9	5.2	5.5	4.9	5.8	5.4	5.7	5.1	5.2	6
Aqueous extract	32.4	42	32	40.3	36	32	40.9	38	36	41
Total nitrogen	5.5	5.3	6.2	5.9	5.9	5.3	5.6	5.9	5.2	5
Ash soluble in water	3.2	2.9	3.1	2.7	3.1	3	3.2	3.3	2.9	3
Ash insoluble in acid	0.2	0.46	0.29	0.33	0.2	0.3	0.4	0.38	0.5	0.2

The following table is quoted from "Food Adulteration" by J. P. Battershall p. 28, as embodying the results of the analyses of samples representing 2414 packages of high quality Indian tea:—

	Minimum.	Maximum.	Average.
	Per cent	Per cent	Per cent
Moisture	5.83	6.32	5.94
Insoluble leaf	47.12	55.87	51.91
Extractive	37.80	40.35	38.84
Tannin	13.04	18.87	15.32
Caffeine	1.88	3.24	2.74
Ash—total	5.05	6.02	5.61
Soluble in H ₂ O	3.12	4.28	3.52
Insoluble in acid	0.12	0.30	0.18

The infusion of tea, as used as a beverage, does not contain the whole of the soluble constituents of the leaf, since the conditions of the extraction are not such as to entirely exhaust the tea.

The following table by Geisler ("Analyst," ix. 221) shows the characters of the infusion made by allowing 100 parts of water to stand for ten minutes on one part of tea. The water was distilled, and heated to boiling-point. The figure "ratio to total" indicates the percentage found in the infusion of that present in the tea:—

Variety of Tea.	Extract.		Tannin.		Caffeine.	Ash.	
	Infusion.	Ratio to Total.	Infusion.	Ratio to Total.	Infusion.	Infusion.	Ratio to Total.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Ceylon Pekoe Tips	33.25	76.6	17.19	75.3	2.44	3.44	91.0
Assam	29.15	73.5	11.48	60.8	3.30	3.80	70.0
"	28.57	72	9.5	58.4	2.75	4.40	79.5
Moyone	37.32	73.2	16.79	87.8	2.95	4.60	55.8
"	28.07	79.4	9.27	77.7	1.67	4.02	66.1
Japanese	31.75	75.6	11.23	74.5	2.17	4.27	80.8
"	34.37	79.6	13.41	94.4	2.07	3.67	63.6
Formosa	33.62	75.9	12.91	75.6	2.50	4.00	71.3
"	33.30	73.7	13.75	68.5	2.42	3.97	66.5
"	29.00	68.6	9.6	59.6	2.12	3.66	62.3
Amoy	27.40	60.9	10.12	56	1.92	3.72	68.5
"	24.50	60.5	7.53	55.6	1.70	3.25	58.9
Moning	24.25	70.6	5.46	41.7	2.87	4.13	73.7
"	21.55	57.8	4.44	32.0	2.77	3.70	63.5
"	21.02	68.6	5.55	45.2	2.33	3.22	58.3
Kaisow	23.25	64.1	4.05	38.5	2.35	3.30	59.9
Moning	19.50	72.2	4.50	52.9	1.95	2.88	46.8

The following figures were obtained at the laboratories of the Imperial Institute for teas grown in the Nyasaland Protectorate and in Natal. In all cases the soluble extract is not the true extractive, but the soluble matter extracted by infusing the tea in 100 times its weight of boiling water for ten minutes:—

No.	Nyasaland Tea.					
	Description.	Moisture.	Ash.	Caffeine.	Tannin determined by Eder's method.	Soluble Extract.
1	" Orange	Per cent	Per cent	Per cent	Per cent	Per cent
4		8.26	5.03	3.68	10.5	26.5
2	" Broken	7.84	5.28	3.54	10.4	25.4
6		8.32	5.09	3.35	9.5	23.7
3	" Mixed "	7.77	5.60	3.22	9.8	23.0
5		8.58	5.20	3.08	10.3	29.6
5	" Dust	8.43	5.17	3.19	10.6	28.8
	" Fannings "					

Estate.	Description.	Moisture.	Natal Tea.			
			Percentages calculated on material dried at 100° C.			
			Ash.	Extract. ²	Caffeine.	Tannin. ³
Kearnsy	Grade 1 ¹	9.1	5.8	26.1	3.9	7.2
"	Grade 2 ¹	7.6	5.6	28.8	3.6	6.3
"	Grade 3 ¹	7.4	5.2	27.4	3.1	6.7
"	Grade 4 ¹	8.7	5.9	25.0	3.4	6.8
"	Flowery Pekoe	7.6	5.1	not determined		7.0
"	Broken Pekoe	6.9	5.8	not determined		7.3
Barnsdale	Pekoe	5.96	5.8	26.2	4.8	10.5
Clifton	} Pekoe	6.2	4.8	31.4	not determined	13.0
Barnsdale						
"	Golden Pekoe	5.5	5.5	28.0	4.4	11.5
"	Flowery Pekoe	6.1	5.3	27.0	4.2	11.6
Aroma	Pekoe Souchong	7.1	5.5	24.3	4.0	10.4
"	Fine Natal Souchong	8.0	5.0	20.9	4.1	10.1
Barrow Green	Choicest Golden Pekoe	7.7	5.2	33.0	4.4	10.8
Average		7.1	5.4	27.1	4.0	9.2

Adulterants of Tea.—The adulterants that have been met with from time to time in tea are either mineral matter added for the purpose (1) of increasing its weight, (2) of causing a more complete extraction of the colouring matter or the tannin, (3) of improving the appearance, by the process of facing; or organic matter added for the same purposes.

Amongst mineral matters, the following have been met with: sand, magnetic iron ore and brass filings (!), sodium carbonate, borax, steatite, and prussian blue. The only organic matters that come into serious consideration are exhausted tea leaves, the leaves of other plants than the tea shrub, prussian blue or indigo for facing, and, rarely, astringent matter such as gambier and catechu to increase the astringency.

THE ANALYSIS OF TEA.

Moisture.—The moisture in the tea should average about 6.8 per cent, rarely up to 10 per cent, and never exceeding 12 per cent.

Mineral Matter.—Five grms. of the sample should be ignited, and

¹ These four samples were taken from specimens in the Natal Court of the Imperial Institute; the remainder were from the South African Products Exhibition.

² "Extractive matter," or "Extract," is the percentage dissolved by treating a given quantity of the tea with one hundred times its weight of boiling water, and allowing it to infuse for ten minutes.

³ Determined by Procter's modification of Löwenthal's process.

the ash examined. The total ash (which is often green owing to the presence of manganese or copper) should be weighed; then the water-soluble ash determined, and lastly the amount of siliceous matter. The alkalinity of the ash should also be determined by titration with decinormal sulphuric acid, using methyl-orange as indicator. The total ash varies from about 5 per cent to 7 per cent, rarely reaching 8 per cent; of this about half is soluble in water. The Society of Public Analysts suggested (in 1874) 8 per cent as the maximum ash of tea which had been dried at 100° of which at least 3 per cent should be soluble in water. The following figures represent the results obtained on 9 samples of tea in the author's laboratory :—

Moisture.	Total Ash.	Soluble in Water.	Siliceous Matter.	Alkalinity as K ₂ O.
Per cent	Per cent	Per cent	Per cent	Per cent
8	7.72	4.0	0.39	2.21
7.5	7.61	3.7	0.41	2.31
9.0	6.04	3.2	0.67	1.87
6.4	6.33	4.15	0.62	1.71
5.8	7.81	4.01	0.24	2.22
8.2	5.42	2.8	0.19	1.28
6.6	6.11	2.79	0.33	1.41
7.1	5.82	2.72	0.71	1.49
8.6	5.91	3.18	0.75	1.81

Sheridan, in a private communication to the late A. H. Allen, gave the following results of the examination of commercial black teas in the Customs Laboratory :—

Tea.	Total Ash.	Ash Soluble in Water.	Siliceous Matter.	Extract.
	Per cent	Per cent	Per cent	Per cent
Indian	5.40	3.20	0.45	40.49
"	6.10	3.30	0.90	29.32
"	5.70	3.15	0.60	39.44
"	5.75	3.25	0.70	38.78
Ceylon	5.50	3.20	0.20	42.90
"	5.40	3.35	0.25	38.24
"	5.60	3.40	0.30	37.98
Japan	6.12	3.15	0.95	29.80
Java	5.60	3.05	0.50	34.60
"	7.65	3.75	1.05	30.72
China	5.70	3.25	0.50	32.95
"	5.85	2.95	1.00	31.71
"	5.60	3.05	0.65	33.57
"	5.65	3.20	0.70	34.10
"	5.45	3.05	0.55	35.70
Port Natal	5.65	3.10	0.45	34.80

The ash of three samples of tea examined by the author contained the following (average) :—

Calcium (as oxide)	9.3	per cent
Magnesium (as oxide)	5.60	"
Fe ₂ O ₃	5.65	"
Manganese (as Mn ₂ O ₃)	1.05	"
P ₂ O ₅	12.25	"
SO ₂	6.41	"
Chlorine	1.86	"
Alkalies (as K ₂ O)	38.5	"
Silica	6.9	"

Wanklyn has given the following figures for the ash of certain other leaves *said* to be used for the purpose of adulterating tea :—

	Total Ash.	Soluble in Water.
	Per cent	Per cent
Beech	4.52	2.0
Bramble	4.53	1.84
Raspberry	7.84	1.72
Hawthorn	8.05	3.78
Willow	9.34	4.16
Plum	9.90	5.66
Elder	10.67	3.19
Gooseberry	13.50	7.83

The following table compiled from analyses by Zoller, Hodges, Wigner, and the author shows the difference between the ash of genuine tea and of exhausted tea leaves :—

	Pure Teas.	Exhausted Leaves.
	Per cent	Per cent
Potash	28.42 to 39.22	6.49 to 7.45
Soda	0.65 " 14.43	0.59
Magnesia	4.40 " 6.47	9.5 " 11.45
Lime	4.24 " 9.3	8.9 " 10.76
Fe ₂ O ₃	2.49 " 5.65	9.2 " 9.8
Mn ₂ O ₃	0.80 " 1.05	1.97 " 2.2
P ₂ O ₅	9.18 " 18.03	20.8 " 25.41
SO ₂	trace to 7.41	trace to 1.8
Cl	0.81 " 3.51	traces
Silica	0.5 " 6.9	7.57 " 9.2

According to Allen the adulteration of tea with magnetic iron filings used to be very common. It is now, however, quite obsolete.

To detect it, 10 grms. of the powdered tea are spread out, and the magnetic particles easily picked up by a strong magnet, washed, dried, and weighed.

Aqueous Extract.—By this is understood the total solid matter

which can be obtained by complete exhaustion of the tea by boiling water. Tea takes a very long time to completely exhaust, and a relatively large amount of water is necessary. To show the necessity of this, the following figures were obtained on the same sample of tea, which was first dried at 100° and then powdered. The tea was boiled under an upright condenser for the time specified :—

Amount of Tea.	Amount of Water.	Time of Boiling.	Extract.
5 grams.	200 c.c.	1 hour	Per cent 25·5
" "	300 "	" "	27·2
" "	500 "	" "	28·4
" "	" "	2 hours	30·0
" "	" "	3 "	31·0

Five grms. should be boiled with 500 c.c. of water for at least two hours under a reflux condenser, and on cooling the liquid should be made up to 500 c.c. again, and 100 c.c. of the clear solution evaporated, and the extract dried in a water oven and weighed. The aqueous extract includes the caffeine, tannin, most of the colouring matter, and various other constituents. The infusion of tea, as made for drinking purposes, does not contain nearly all the soluble matters, and the properties of the ordinary infusion are only of importance in gauging the character of a tea as used in practice, a complete extraction being necessary in dealing with questions of adulteration. The table of Geisler's results (page 4) gives the average values of the total extract and of that of an infusion prepared by pouring on the leaves 100 times their weight of boiling water, and allowing them to infuse for ten minutes.

The extract obtained by complete exhaustion varies from about 32 per cent up to as much as 40 per cent in certain classes of tea. It is obvious, therefore, that the presence of exhausted leaves will not necessarily be indicated if the original leaves present contain a very high amount of extract. Green tea yields a rather higher extract than black tea, the lowest permissible limits being 30 per cent for black tea and 38 per cent for green tea.

Tatlock and Thomson ("Analyst," xxxv. 103) prefer to boil 1 gm. of tea with 400 c.c. of water for one hour under a reflux condenser, collect the insoluble matter, wash with 80 c.c. of hot water and dry and weigh. The weight of the insoluble matter, plus the moisture, deducted from 100 gives the percentage of water-soluble ingredients.

They give the following limits for a number of samples :—

	Per cent
Indian teas	43·47 to 49·75
Ceylon teas	41·32 „ 48·25
China teas	38·43 „ 46·94

Ordinary exhausted tea leaves, that is the residues from restaurants, etc., contain about 25 to 30 per cent of their original extractive matter.

The following are results obtained by various observers with teas of specified characters:—

		Kenrick. ¹	Wigner.	Peligot.	Parry.
		Per cent	Per cent	Per cent	Per cent
Congou	teas	23·37	26 to 33 ²	36·8	31 to 38
Assam	"	38·53	33·3	41·7	32 „ 42
Hyson	"	34·22	36·8	43·8	—
Ceylon	"	27·45	—	—	29 to 37
Gunpowder	"	28·55	33 to 40	48·5	—
Japan	"	30·07	—	—	—
Pekoe	"	—	34·2	38·0	37
China	"	—	—	—	29 to 41·5

Caffeine.—This may be determined by the methods described under coffee (page 38), but it is preferable to use 5 to 6 grms. of tea for the determination. The percentage of caffeine varies between 2·5 per cent and 4 per cent, rarely falling below 2·9 per cent. Many of the older figures given by various observers are due to the fact that the processes adopted (as is now recognized) gave too low results.

Tatlock and Thomson (*supra*, loc. cit.) prefer to determine caffeine by boiling 2 grms. of the tea under a reflux condenser with 800 c.c. of water for an hour. The filtered liquid is evaporated to 40 c.c., cooled, mixed with 10 c.c. of normal alkali, and extracted with three successive quantities of 40, 30 and 10 c.c. of chloroform. The mixed chloroformic liquids are washed with 10 c.c. of normal alkali, then with water and finally the chloroform evaporated and the caffeine weighed.

Burmah ("Bull. Soc. Chim." 1910, [iv.], 239-44) gives the following method for the determination of caffeine, which he found to give more accurate results than any method hitherto adopted, including that of Keller, which was found inapplicable to roasted coffee. Five grms. of the finely powdered sample are dried and freed from fat by extraction with petroleum spirit, and the residue shaken for some minutes in a stoppered flask with 150 grms. of chloroform after which 5 grms. of a 10 per cent solution of ammonia are added and the shaking continued for 30 minutes. The liquid is then filtered quantitatively into a tared Erlenmeyer flask, the solvent expelled and the residue of crude caffeine heated in the water oven until constant in weight. It is next dissolved in a little chloroform and introduced into a test tube (150 to 280 mm. in length and 15 to 18 mm. in diameter) which has a constriction near the bottom and another near the top. The solvent is evaporated from the tube, which is then dried at 100° C. or "in vacuo". The lower constriction is now closely packed with a plug of asbestos wool, while the portion above the upper constriction is filled with cotton wool. The bottom of the tube containing the crude caffeine is then immersed in melted paraffin, the temperature of which is maintained at 210° to 240° C. and, after three hours heating, the whole of the caffeine will have

¹ By ten minutes infusion.

² On the dried tea.

sublimed and condensed in the portion of the tube between the constrictions. This portion may then be cut off with a file and the caffeine dissolved from it with a little chloroform, which is subsequently evaporated. The weight of the dried residue gives the amount of pure caffeine. In the case of tea, it is not necessary to exhaust with petroleum spirit.

Tannin.—The determination of the tannin in tea is of the greatest value, inasmuch as an excess indicates the presence of added astringent matter, whilst a low tannin value indicates the presence of either exhausted tea leaves or leaves of some other plant which contain but little tannin. The tannin present in genuine tea varies from 9.0 per cent to about 18 per cent, the average being almost 12 per cent. Any quantity less or more than these limits must be regarded with suspicion.

Janke (using the copper acetate process described below) found, with eighteen samples, 6.9 per cent as the lowest, and 9.2 per cent as the highest limit in black tea, whilst green tea gave from 8.5 per cent to 9.9 per cent (but see below). Exhausted tea leaves—that is tea leaves infused for the beverage—contain from 1.5 to 4 per cent of tannin.

Very many processes have been described for the determination of tannin, none of which can be said to yield absolutely accurate results, and in reporting on the amount of tannin present, it is necessary, in order to compare results by different analysts, to state the process used.

The most reliable only of the many processes described will be here discussed.

(1) The copper acetate process. About 2 grms. of finely powdered tea are extracted by boiling for an hour with two successive quantities of 100 c.c. of water. The filtered extracts are heated to boiling and then 30 c.c. of a 5 per cent solution of cupric acetate are added. The precipitate, consisting of a tannate of copper, is collected on a filter, washed, and ignited, the copper ash being fully oxidized by the addition of a few drops of nitric acid. One part of CuO may be regarded as equivalent to 1.305 part of tannin. This process is due to Eder (*"Ding. Poly. Journ."* 129, 81). By this process Eder found an average of 10 per cent of tannin in black tea, and 12.3 per cent in green tea.

(2) Lead acetate process. Fletcher and Allen (*"Chemical News,"* 29, 169 and 189) proposed the use of acetate of lead for the determination of tannin. This process, which includes the small amount of gallic acid present, is carried out as follows: An infusion is made by completely extracting 2 grms. of tea, in the same manner as for the determination of the total extract, the liquid being made up to 250 c.c. Three quantities of 10 c.c. of a 0.5 per cent solution of neutral acetate of lead are placed in beakers, each being diluted to about 100 c.c. with boiling water. The process is a titration one, the indicator being a solution of .1 gm. of potassium ferricyanide in 100 c.c. of water and 100 c.c. of strong ammonia of specific gravity 0.880. This indicator gives a deep red colour with tannic or gallic acids. The standard tea infusion (2 grms. in 250 c.c.) is run in from a burette into the three trial quantities of lead acetate solution. The first beaker should receive 12, the second 15 and the third 18 c.c. of the tea infusion (if green tea is being examined 8, 10 and 12 c.c. will be sufficient). After well

stirring, a few drops of the liquid are filtered and allowed to fall on to a few drops of the indicator on a porcelain slab. In the presence of tannin a pink colour will be observed. The approximate quantity of the infusion will be easily determined by this preliminary experiment, and a fresh titration is now carried out, and the approximate amount of tea infusion at once run in. If tannin is still indicated another small addition of the infusion is made and a few drops again filtered and tested. According to Allen 10 c.c. of a 0.5 per cent acetate of lead solution will precipitate 0.010 grm. of pure tannic acid. So that if the above-described quantities be adhered to, the number of c.c. of tea infusion divided into 125 will give the percentage of tannin in the sample. Fletcher and Allen thus found 8.5 to 11.6 per cent of tannin in black tea. Catechu tested by this method gives a result of 105 to 125 per cent of tannin. It is therefore obvious that the method is only comparative, and not absolute.

(3) Löwenthal's process. This process depends on the oxidation of the tannin by means of potassium permanganate. In principle the process is as follows: Tannin is much more easily oxidized by potassium permanganate than indigo. It is, however, impossible to determine the end of the reaction when a coloured solution of tannin is titrated with the permanganate. With indigo, however, the end reaction is comparatively sharp. A known quantity of indigo solution is, therefore, added to the solution of tannin from a given quantity of tea, and the amount of permanganate required for oxidation of the tannin and the indigo is noted. As the tannin is oxidized first, the end reaction is that of the indigo and is fairly sharp. The same quantity of the indigo solution is now oxidized alone, and the quantity of permanganate used is deducted from the former figure, by which the amount necessary to oxidize the tannin is ascertained. But besides the tannic acid (in whatever form it is present), the gallic acid and certain other substances are also oxidized. A known volume of the extract of the tea is therefore heated with gelatine solution to precipitate the tannic acid, and the filtrate, together with a known quantity of indigo solution, is again titrated with permanganate solution. The quantity used for the oxidation of the gallic acid, etc., is deducted from the amount required for the "total astringent matter" and the amount used for the tannin is thus ascertained. The tannin is now determined in terms of potassium permanganate. But as it is uncertain in what form all the tannic acid of tea is present, the conversion of the permanganate figure into tannic acid must be an empirical matter.

In practice the determination may be carried out as follows (Procter's modification). The solutions necessary are:—

- (i) A solution of potassium permanganate of about 1 grm. per litre.
- (ii) A solution of indigo, containing 5 grms. of pure indigo-carmin (sodium sulphindigotate) and 50 c.c. of strong H_2SO_4 per litre.
- (iii) A solution of gelatine (2 per cent strength).

The extract of the tea used for determining the amount of water-soluble extract may be used for the determination (1 in 100).

Löwenthal gives the following concrete example, as illustrating the calculation necessary:—

A 1 per cent solution of the extractive matter of sumach was used. Ten c.c. were diluted with 75 c.c. of water and 25 c.c. of the indigo solution and 10 c.c. of dilute sulphuric acid were added. The potassium permanganate is slowly run in until the blue colour changes to yellow, when reaction is considered to be complete. The same amount of indigo and acid are now titrated and the result noted. For the most concordant results, the indigo should require about twice as much permanganate as the tannin. 100 c.c. of the watery extract are then mixed with 50 c.c. of the gelatine solution, and the mixture is well stirred; 100 c.c. of the salt solution are then added and the whole allowed to stand for twelve hours. A portion of the liquid is now filtered and the same process is repeated in an aliquot portion of the filtrate, which is, of course, now deprived of tannic acid. For example:—

10 c.c. of the extract	}	required	16.6 c.c. of permanganate solution.
25 c.c. of indigo solution			
25 c.c. of indigo solution			6.6 c.c.
∴ required for the "tannin" only			10.0 c.c.
25 of the filtrate from the gelatine	}	"	11.2 c.c.
(= 10 c.c. original extract)			
and 25 c.c. indigo solution			
but 25 c.c. indigo solution alone			
		"	6.6 c.c.

Therefore the gallic acid, etc., required 4.6 c.c. But as the total astringent matters, or "tannin," required 10.0 c.c., it follows that 5.4 c.c. of the permanganate solution were used by the tannic acid and 4.6 c.c. for the gallic acid, etc.

The best quantities of the extract of tea leaves (1 per cent) to use, are 4 c.c. for the titration in the first instance, and 8 c.c. of the extract after treatment with gelatine.

The titration should be carried out as follows:—

The extract (corresponding to 0.04 gm. of tea) is diluted to about 500 c.c. with water and 20 c.c. of the indigo solution added. The solution of potassium permanganate is then run in slowly with vigorous stirring until the liquid is transparent, when the permanganate is run in very cautiously and slowly until the yellow solution appears of a faint pink colour in the margin. A second titration in an equal quantity is made and the two results, representing 0.08 gm. of tea, are added together (a).

The same titration is then made on 40 c.c. of the indigo solution without the tea extract. The amount of permanganate used (b) is deducted from a. Now (a - b) represents the amount of permanganate used to oxidize the tannin and other similar matter in the tea. If (a - b) is more than two-thirds of b, correct results will not be yielded and the amount of indigo must be adjusted accordingly.

The extract of the tea corresponding to 0.080 gm. is then mixed with about 25 c.c. of the gelatine solution and the mixture is saturated with ordinary salt. 10 c.c. of 10 per cent sulphuric acid are added and the whole diluted with water. The liquid is now well shaken with a little kaolin and filtered; the precipitate can either be well washed with water, or an aliquot portion of the filtrate, after the liquid has been made up to a definite volume, can be used and the necessary calculation

made. The filtrate is now mixed with 40 c.c. of the indigo solution and titrated as before. The permanganate solution used is that required to oxidize the indigo and the oxidizable matter of the tea other than tannin (*c*). Hence $a - c$ represents the permanganate used to oxidize the tannin.

It must be remembered that no estimation of tannin is absolute, but is comparative as between determinations on the same material. For example, the tannin of tea and that of, say, sumach, require different quantities of permanganate for oxidation. Hence it is very common to report the tannin value of tanning materials in terms of permanganate, or rather of oxalic acid, which is quantitatively oxidized at once by permanganate.

If 10 c.c. of a decinormal solution of oxalic acid be titrated with the permanganate solution in the presence of a little dilute sulphuric acid, the volume of permanganate required is that which will oxidize 63 milligrams of oxalic acid.

The above results, therefore, enable the tannin to be expressed in terms of crystallized oxalic acid.

Any attempt to return the actual amount of tannin, obviously requires the knowledge of the reducing power of the tannin as compared with that of oxalic acid. The term "reduction equivalent" is used to indicate the weight of tannin that will reduce the same amount of potassium permanganate as 63 grms. of oxalic acid (a normal solution contains 63 grms. per litre). The actual reduction equivalent of tea tannin is a matter not yet settled, but it is generally believed to be practically identical with that of oak bark tannin, which is 62.3. So that excellent comparative results may be obtained by calculating as though tea tannin possessed the same reducing power as oxalic acid.

(4) Vignon ("Comptes Rendus," cxxvii. 369) has suggested the following simple method for an approximate determination of tannin. An extract is made which contains about 0.1 per cent of tannin—say 1 gm. of tea to 150 c.c. of water. The total solid matter is determined in a portion of this and then 5 grms. of pure white silk free from any dressing is placed in the liquid, which is kept at 50° C. and occasionally shaken for two hours. The silk absorbs the tannin, and the difference between the total solids before and after the treatment with silk is returned as tannin.

(5) Gelatine process. An approximate estimation of the tannin may be made by titrating a definite volume of the extract with a 2 per cent solution of gelatine to which a trace of alum has been added. The gelatine solution is standardized by a solution of tannin of known strength. The tannin solution should be titrated with the gelatine solution until no further precipitation occurs. The exact ending of the titration may be observed by allowing the precipitate to settle after each addition of gelatine solution, and then placing a few drops of the clear liquid in a watch glass and testing with gelatine solution to ascertain if precipitation be complete. The tannin value of the gelatine solution being known, the tea extract is titrated in the same manner.

(6) Hide powder. The tannin is absorbed by hide powder, and the difference in the solid matter of the extract before and after

absorption gives, approximately, the amount of tannin. An extract of tea is made so as to contain about 1 per cent of tannin (preferably about 8 grms. of tea thoroughly exhausted and the extract concentrated to 100 c.c.). Procter operates as follows:—

A piece of glass tubing about 4 inches long and 1 inch in diameter is packed tightly, but without ramming down, with finely powdered pure hide powder. A siphon tube is inserted into the cork which closes the upper end of this tube, the opening of which is lightly plugged with a piece of cotton wool and covered with muslin. The lower end is covered with fine muslin held in position by an india-rubber band. The hide powder tube is now immersed in 100 c.c. of the extract, of which the total solid matter has been determined, and after the hide powder is thoroughly well soaked, the siphon is started by suction and 50 c.c. of the liquid collected; the lower end of the siphon should be connected with an india-rubber tube and burette clip, so as to regulate the flow. This should be slow, so as to ensure the absorption of the tannin. The difference between the solid matter in the extract that has passed through the hide powder is returned as tannin. A blank experiment should be made with hide powder and distilled water (as there is always a little soluble matter in the hide powder) and the necessary correction made.

Tatlock and Thomson (*vide supra*; loc. cit.) prefer to determine tannin by precipitating the aqueous extract by a slightly acidified solution of quinine sulphate, and to wash and dry the quinine tannate, which is stated to contain 75 per cent of its weight of tannin. The least variation in the conditions of the experiment will, however, cause the composition of the precipitate to vary.

Allen recommends the following for the detection of catechu in tea, but states that the suspected sample should be tested side by side with a sample of genuine tea. One grm. of pure tea, and an equal quantity of the suspected sample, are infused in 100 c.c. each of boiling water, and the strained liquids precipitated while boiling with a slight excess of neutral lead acetate; 20 c.c. of the filtrate from pure tea, which should be colourless, when treated with a few drops of silver nitrate solution gives only a greyish colour, and if cautiously heated gives only a very slight greyish cloud or precipitate of reduced silver. But in the presence of 2 per cent of catechu, there will be a copious precipitate of a brownish colour, the liquid becoming decidedly yellow.

It has been said that foreign leaves are legitimately present in tea to the extent of 1 or 2 per cent, being added in order to impart a special bouquet to the tea. It is more probable, however, that any foreign leaves added to tea for this purpose are removed after imparting such bouquet to the tea. According to Wynter Blyth, a crystalline sublimate, which he considers to be caffeine, is obtainable from every single leaf of tea. A leaf is boiled for a minute in a watch glass with a very little water, a little magnesium oxide added and the liquid evaporated down to a single drop. This is transferred to a microscopic cover glass and evaporated almost to dryness on a hot plate. It is surrounded by a glass ring and on evaporation of the last drop of water, a second cover glass is placed on the ring. In the presence

of caffeine, further heating causes a crystalline sublimate to collect on the upper glass which is clearly visible under the microscope. Certain other leaves yield a sublimate, but according to Blyth, if no sublimate is found, the leaf is not that of tea. The same chemist has also claimed that the presence of manganese in tea leaves is a certain method of recognizing them. The ash of a leaf is taken up on a bead of sodium carbonate on a platinum looped wire, and on fusion with a trace of KNO_3 the green colour of potassium manganate is found. Allen has found manganese in the leaves of *Camellia Japonica*, *Camellia sasanqua*, *Coffea Arabica*, the beech, blackberry and sycamore. Other leaves examined showed either no manganese, or only faint traces.

Structural and Microscopic Examination.—Some of the leaves should be soaked in hot water. The facing, if any be present, as used to regularly be the case with green teas, will become detached, and a little may be examined under the microscope if necessary, and will be found to be structureless. The bulk of the facing will sink to the bottom of the water, or may be collected and examined. Indigo may be recognized by giving a yellow colour with a drop of nitric acid; prussian blue is detected by heating with caustic alkali, acidulating with hydrochloric acid, and testing for ferrocyanide by ferric chloride. Any matter left insoluble after warming with caustic alkali, should be then treated with hydrochloric acid; any insoluble matter will usually be of a siliceous nature, such as steatite.

The venation and serration of the leaf can to some extent be observed on the leaf which has been soaked in hot water and dried between blotting paper, by means of a lens. Blyth proposes cleaning the leaf by warming with a strongly alkaline solution of potassium permanganate; sodium hypobromite is also useful. The leaf should be kept between two cover glasses, the upper one being kept in position by a small weight. The leaf, previously soaked in the alkaline permanganate, is then washed in water, treated with HCl , again washed and then examined. The details of its structure can then be examined. A better method is to infuse the leaves in boiling water twice. Remove superfluous moisture carefully by means of blotting paper. The leaves should then be immersed for ten days in a 70 per cent solution of chloral hydrate. They can then be conveniently examined.

A leaf will be found to be bi-facial; the epidermis, both upper and lower, can be examined in a transverse section and will be found to consist of small cells, and if the sections are suitable, characteristic long hairs will be found. One or two rows of palisade parenchyma will be found, and the spongy parenchyma with large air spaces. In the centre of the leaf numerous cells are found containing crystals of calcium oxalate in various form. The principal diagnostic characters are as follows:—

- (1) The long hairs with a radiate arrangement of cells at their bases.
- (2) The rosette crystals of calcium oxalate.
- (3) Peculiar sclerenchymatous cells, known as idioblasts, found in the mesophylllic tissues. These are most common in the midrib and petiole.

The walls of these idioblasts are highly lignified and are well stained by phloroglucinol and hydrochloric acid.

An examination of the surface preparation is also very useful. The venation consists of well-defined loops, not found in leaves likely to be met with as adulterants. Each of the serrations is a more or less hook-shaped tooth consisting of a conical mass of parenchymatous cells. They often fall off on old leaves, leaving a characteristic scar. The under surface has numerous oval stomata with characteristic spaces between the guard cells.

The hairs are very long—although often absent on old leaves. They are unicellular, and very thick-walled, and are generally bent nearly at right angles near the base, so as to lie almost flat on the leafy surface.

In examining tea leaves in this manner, the surest guide is to consider the above-described characters side by side with leaves of known authenticity. The comparative rarity of cases in which one finds other leaves present does not justify the space that would be necessary for a description of the botanical or microscopical characters of leaves said to be used as adulterants of tea. No such leaf shows the characters described above and a comparison of suspected samples with genuine tea leaves will at once demonstrate whether the suspected sample is tea or not. Identification of foreign leaves can only be definitely made by comparison with similar leaves of known origin, and is very rarely necessary.

COCOA AND CHOCOLATE.

Cocoa of commerce consists of the slightly roasted seeds of several species of *Theobroma*—principally *T. cacao*. The seeds consist of the shells or husks, and the cotyledons or kernels, the latter being known as cocoa nibs, and these when ground, after being freed from the husks, constitute what is understood as cocoa. Chocolate is understood to mean cocoa both sweetened and flavoured (principally with vanilla), but no legal standard appears to exist for what chocolate should be, hence its composition is very variable. This will be dealt with later.

The average compositions of (1) natural cocoa, (2) cocoa nibs, and (3) cocoa husks, are given in the following tables which are the results of numerous analysis by König, Heisch and others, together with samples examined by the author:—

(1) COCOA WITH THE HUSKS (ROASTED).

Moisture	5	to	8	per cent
Fat	40	"	50	"
Carbohydrates	10	"	14	"
Ash (total)	3	"	4.6	"
Ash (water-soluble)	1.5	"	2.4	"
Nitrogen	1.7	"	2.4	"
Theobromine	1	"	2	"

(2) ROASTED COCOA NIBS.

Moisture	3.5 to 4.5	per cent
Fat	45 " 55	"
Carbohydrates	10 " 14	"
Ash (total)	2.3 " 4.0	"
Ash (water-soluble)	0.9 " 1.8	"
Nitrogen	1.6 " 2.15	"
Theobromine	1.5 " 2.5	"
Cellulose	3 " 4.5	"

(3) COCOA HUSKS (ROASTED).

Moisture	3 to 8	per cent
Fat	4 " 5	"
Carbohydrates	7 " 10	"
Ash (total)	6 " 20	"
Nitrogen	1.5 " 2.4	"
Cellulose	13 " 18	"

The following tables are given by Booth, Cribb and Richards, ("Analyst," xxxiv. 137).

	Granada Bean (with husk).		Trinidad Bean (without husk).	
	Raw.	Roasted.	Raw.	Roasted.
	Per cent	Per cent	Per cent	Per cent
H ₂ O	6.32	3.10	6.67	4.45
Fat	46.50	49.96	54.60	55.70
Nitrogen	1.96	1.86	2.28	2.32
Fibre	3.60	3.90	2.45	2.48
Ash	2.86	3.12	2.87	2.73
Siliceous matter	0.10	0.12	0.03	0.08
Soluble ash	1.26	1.44	0.94	0.95
Alkalinity as K ₂ O	0.68	0.75	0.42	0.43
Cold water extract	13.50	12.90	12.73	12.00

ANALYSIS OF NIBS OF KNOWN ORIGIN.

	Ash.	Soluble Ash.	Siliceous Matter	Alkalinity of Ash as K ₂ O.	Cold Water Extract.	Nitrogen.	Fat.	Fibre.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
African	2.52	0.98	0.05	0.38	11.58	1.84	50.2	—
Granada	2.60	1.04	0.03	0.55	9.8	2.26	50.8	2.97
Guayaquil	3.16	1.32	0.04	0.53	11.4	—	—	—
Trinidad	2.73	0.95	0.08	0.44	12.0	2.32	55.7	2.48
Caracas	3.24	1.58	0.08	0.74	—	—	—	—
Bahia	2.68	1.22	0.05	0.51	9.5	1.98	44.4	—
Acera	3.22	1.36	0.04	0.41	11.4	2.46	50.6	2.87
Ceylon	3.81	1.66	0.03	0.67	11.9	2.44	50.2	2.36
Para	3.22	1.14	0.06	0.45	12.1	—	—	—
Puerto Cabello	3.88	1.50	0.13	0.64	12.6	2.35	51.3	3.02

The ash of cocoa husks contains a variable amount of matter insoluble in acid, which is not the case with the ash of the cocoa nibs.

ANALYSIS OF HUSKS FROM KNOWN SOURCES.

	Ash.	Soluble Ash.	Siliceous Matter.	Alkalinity of Ash as K ₂ O.	Cold Water Extract.	Nitrogen.	Fat.	Fibre.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Ceylon	6.61	4.78	1.0	2.54	20.7	2.4	3.1	12.8
African	5.63	3.53	1.79	2.63	20.4	2.91	3.5	15.7
Para	6.78	4.39	0.72	2.80	18.7	—	—	—
Guayaquil	8.19	5.25	1.45	3.36	24.6	2.13	5.9	12.85
Puerto Cabello	20.82	5.24	8.33	1.13	23.5	—	5.68	13.83
A	8.48	5.78	0.82	2.51	19.5	2.76	3.31	15.8
B	11.68	4.08	3.34	2.24	20.3	—	—	—
C	16.28	—	—	—	18.9	2.29	4.62	14.80
D (raw)	7.82	4.62	0.86	2.12	24.4	—	—	—

The following table is due to Ridenour ("Amer. Jour. Pharm." 1895, 207):—

	Bahia.	Surinam.	Java.	Trinidad.	Aruba.	Caracas.	Granada.	Roasted Trinidad.	Tobasco.	Roasted Caracas.	Machalle.	Maracaybo.	Average.
Fat (cacao butter)	42.10	41.03	45.40	43.66	43.31	36.81	44.11	41.89	50.95	37.63	46.84	42.20	42.99
Theobromine	1.08	.93	1.16	.85	.86	1.13	.75	.95	1.15	.99	.76	1.03	.97
Albuminoids	7.50	10.54	9.25	11.90	10.14	10.59	9.76	12.02	7.85	12.36	12.69	11.56	10.51
Glucose	1.07	1.27	1.23	1.38	.42	2.76	1.81	1.48	.94	1.76	1.60	1.09	1.40
Saccharose	.51	.35	.51	.32	6.37	1.56	.55	.28	2.72	.51	.46	1.36	1.29
Starch	7.53	3.61	5.17	4.98	1.58	3.81	6.27	5.70	3.51	6.07	1.35	1.69	4.27
Lignin	7.86	3.90	6.10	5.65	4.62	3.28	5.55	5.87	6.44	9.05	5.95	7.16	5.95
Cellulose	13.80	16.24	13.85	13.01	14.07	16.35	13.49	19.64	12.57	11.69	11.32	17.32	14.44
Extractive matter (by difference)	8.99	13.53	8.90	8.31	9.00	12.72	9.72	5.84	9.26	9.22	9.02	6.79	9.30
Moisture	5.96	5.55	5.12	6.34	5.90	6.63	5.28	2.63	1.55	5.69	5.86	5.67	5.18
Ash	3.60	3.05	3.31	3.60	3.73	4.36	2.71	3.70	3.06	5.03	4.15	4.13	3.70

The following figures represent average values for the nibs and husks :—

	Nibs.	Husks.	Dry and Fat-free Nibs.	Dry and Fat-free Husks.
	Per cent	Per cent	Per cent	Per cent
H ₂ O	3.0	4.5	—	—
Ash	3.07	7.3	6.14	7.84
Siliceous matter	0.05	1.11	0.1	1.2
Fat	50.0	4.4	—	—
Fibre	2.8	14.0	5.9	16.5
Nitrogen	2.5	2.5	5.05	2.54
Cold water extract	11.6	22.0	24.2	23.6

The following figures are those of certain flours sometimes found as adulterants :—

	Cold Water Extract.	Nitrogen.	Ash.
	Per cent	Per cent	Per cent
Wheat	7.7	1.97	0.5
Barley	5.1	1.2	0.9
Maize	0.8	0.14	0.4
Rice	0.9	1.23	0.5
Sago	1.98	0.03	0.14
Arrowroot	0.4	0.13	0.3
Banana	1.5	0.8	2.0

The best cocoas should be made from the nibs only, but the husks are frequently ground with the nibs in the preparation of inferior qualities. So long as the husk is not in excess of that natural to the bean, it may be properly sold as cocoa. In commerce there are two varieties of (prepared or soluble) "cocoa" regularly to be met with. (1) This consists of the ground nibs, with frequently some of the fat removed; (2) preparations to which sugar and frequently starch have been added.

The fat is frequently partly removed, as excess of this constituent renders the manipulation of the cocoa difficult, and its removal renders the product more easy to digest. A small amount of alkali is often added by certain makers, in order to soften the fibre, and to emulsify the fat, so as to render the product more easily miscible.

Adulterants—or considering the legal aspect of the matter—dilutents may be a better term, of cocoa are usually starch and sugar, although other substances are sometimes met with. Brick dust, oxide of iron, iron-earth, chalk and similar substances are given as adulterants in many books—but they are rarely met with—although a little iron preparation may occasionally be added for colouring purposes. Aniline

dyes are also sometimes added. Any organic powder will usually be detected microscopically.

THE ANALYSIS OF COCOA.

Ash.—After determining the moisture (if considered necessary) the ash should be determined. This should be very little in excess of 4 per cent, usually less, unless a notable amount of husk is present, or some inorganic adulterant has been added. The ash should be wholly soluble in hydrochloric acid, but if much cocoa husk be present, a small proportion will be insoluble. The ash of pure cocoa contains from 30 to 50 per cent of phosphoric acid. The alkalinity of the ash varies enormously on account of the addition of a trace of alkali in the preparation of the cocoa, so that it is practically only a measure of the amount of alkali so added, and gives no further information. The presence of sugar and starch naturally diminishes the amount of ash.

Fat.—This should be determined on 10 grms. of the sample, after drying to remove moisture. It is best mixed with sand and extracted in a Soxhlet tube with anhydrous ether. In cases of cocoa to which alkali has been added a little of the fat remains undissolved, being fixed in the form of soap. A trace of acid is necessary to decompose this. The fat should have the character of pure cocoa butter, as described on p. 26. This is important in the case of chocolate and chocolate creams, as many other fats are used to add to cocoa after the more valuable cocoa butter has been extracted for sale as such. The fat in ordinary powdered cocoa varies considerably—if the cocoa is in the natural state usually between 45 per cent and 55 per cent, but usually 20 to 28 per cent is found in cocoa which has been more or less defatted before put on the market.

Sugar.—The dried residue, after the fat has been exhausted, is exhausted with hot alcohol of specific gravity about 0.850. The hot alcoholic solution is heated with a strong solution of lead acetate, which precipitates tannates, tartrates, etc. The alcohol in the filtrate is driven off, and the excess of lead removed by the addition of a strong solution of sodium phosphate. The liquid is now ready for the sugar determination. This is effected either by a polarimetric reading or by inversion and reduction of Fehling's solution, as described under "Sugar" (p. 122). The alcoholic extract of the cocoa has practically no reducing power on the copper solution, so that the whole of the sugar found may be approximately credited to added cane sugar.

Albumenoid Nitrogen.—The residue, after the extraction with ether and alcohol contains the starch and albumenoids, together with cellulose, fibre and gum, etc. After weighing this residue, an aliquot portion may be used for determination of the albumenoid nitrogen by Kjeldahl's method, and this may be multiplied by 6.25 and returned as albumenoids.

This residue is in a suitable condition for microscopic examination, and if any foreign starch is detected it may then be estimated.

Starch.—A weighed portion of the residue which has been extracted with ether and alcohol is heated for an hour with 50 c.c. of a 2 per cent solution of hydrochloric acid at a pressure of two atmospheres (this is

conveniently done by effecting the conversion in a soda water bottle with an india-rubber cork tightly wired in; the bottle is heated to 120° C. in an oil bath which will correspond with the necessary pressure). The starch is now completely converted into dextrose and this is determined by the reduction of Fehling's solution, ten parts of dextrose representing nine parts of starch.

An alternative method of determining the starch present consists in extracting the fat-free sample with cold water, and washing the residue with a .04 per cent solution of caustic soda to remove the albumenoids. The residue is rinsed off the filter with *warm* water, the starch gelatinized by heating, and the liquid heated with a measured quantity of a freshly prepared cold infusion of malt, whose specific gravity has been ascertained. The liquid is kept at 60° to 63° C., with occasional stirring, until the conversion of the starch is finished, as shown by a drop of the liquid giving no blue or brown colour with iodine solution on a white tile. The solution is filtered, made up to a definite volume and the specific gravity taken. From the excess of the specific gravity over 1.000, is subtracted the density due to the solids in the malt infusion—allowing of course for the increase in volume—and the remainder represents the increase in specific gravity due to the starch dissolved. This figure divided by 4.096 gives the number of grms. of starch in 100 c.c. of the solution being examined. For example (as calculated by Allen) if 20 grms. of cocoa be taken, and the solution of gelatinized starch be made up to 50 c.c., and 5 c.c. of infusion of malt of specific gravity 1.060 be added: the liquid is made up eventually to 100 c.c. and is found to have a specific gravity 1.023. The correction for the malt infusion will be $\frac{(1060 - 1000)}{100} \times 5 = 3$. Now the density of

the solution $1023 - 1000 = 23$, and this figure - 3 (the malt density value) = 20. This, divided by 4.096 gives 4.9 grms. per 100 c.c. or 24.5 per cent of starch on the sample.

Cellulose and Fibre.—The mixed cellulose, fibre, and siliceous matter left after the above treatment is washed with 2 per cent caustic alkali solution, then with dilute HCl, alcohol, and finally ether, and dried and weighed. A direct determination of the crude fibre, which is of value as indicating the presence of cocoa husk, can be made by removing the fat from 2 grms. of the sample, and then boiling the residue for half an hour under a reflux condenser with 200 c.c. of water and 2.5 c.c. of strong sulphuric acid. The liquid is filtered through fine linen and the undissolved matter washed with hot water several times and then boiled with 200 c.c. of water and 2.5 grms. of caustic soda. The residue is washed again with hot water, and then with alcohol and finally with ether, dried at 110° and weighed. This—after deducting the ash left on ignition—is to be returned as crude fibre. In cocoa free from husk it will generally vary between 3.5 and 5 per cent, but will be higher than this if husk is present. (Allen, "Commercial Organic Analysis," 2nd edition, Vol. III, part ii, p. 567). This process gives good results, and with the omission of the alcohol and ether washing, is officially used in the United States.

Booth, Cribb, and Richards ("Analyst," xxxiv. 141) remark that in

the course of this process as applied to the analysis of chocolate it is worth while to observe the tint of the solution obtained by the acid treatment, as, if it be only a faint red, there is probably only a very little cocoa present.

Estimation of Husk in Cocoa Powders.—A. Goske ("Zeit. Untersuch. Nahr. Genussm." 1910, 19, 154-8). To carry out the following process the husk is first separated from the cocoa powder by means of calcium chloride solution, advantage being taken of the greater sp. gr. of the husk. Extract 5 grms. of the cocoa powder with ether for sixteen hours, and weigh the amount of fat extracted. Dry the fat-free powder, and mix one gramme of it in a tube with 20 c.c. of calcium chloride solution, prepared by dissolving 107.1 grms. of calcium chloride in 100 c.c. of water, this solution having a sp. gr. of 1.535 at 30° C. Warm the calcium chloride solution to a temperature of about 50° C. before adding the cocoa, thoroughly mix together, and heat to boiling for two minutes. Submit the tube and its contents to centrifugal action for six minutes while still hot. Use a glass rod to break down the froth, then decant the liquid portion from the almost solid sediment. Collect the latter on a weighed filter, wash until free from chloride, dry at 100° C., and weigh. Several samples of commercial husk when submitted to this process yielded from 15 to 38.7 per cent of sediment, the average being 24.5 per cent, calculated on the dry, fat-free substance. The author suggests the use of the highest figure, 38.7 as a standard in ascertaining the amount of husk present in a sample of cocoa. One gramme of dry, fat-free cocoa, for example, yielded 0.0618 gm. of sediment, corresponding with 0.16 gm. of husk, or 13 per cent on the original cocoa, which contained 18.4 per cent of fat. When 6 per cent was subtracted as the amount yielded by ordinary cocoa, the sample contained 7 per cent of added husk.

Total Nitrogen.—Two or three grms. may be used for a determination by Kjeldahl's method. It must be remembered that theobromine contains 31.1 per cent of nitrogen, so that if the albumenoid nitrogen is required, the theobromine must be removed by exhaustion with ether, alcohol, and chloroform, and the nitrogen determined on the residue.

Theobromine.—The principal alkaloid of cocoa is theobromine, $C_7H_8N_4O_2$, but a little caffeine is also present. The amount of alkaloid present in cocoa averages 1.4 to 1.8 per cent, the husks containing a very small quantity. It is not usually necessary to determine the amount of alkaloid present, as, if a cocoa is pure, the alkaloidal value will only be an indication of its quality—and that probably only as a stimulant. Numerous methods have been proposed to determine this value, and in the author's experience that of Beckurts is, on the whole, the most satisfactory. A mixture of 10 grms. of the powdered cocoa and 10 grms. of fine sand is heated on the water bath with 150 c.c. of water and 0.1 c.c. of strong hydrochloric acid, with repeated agitation. After this has been done for about an hour, the fat is allowed to solidify and the aqueous solution of alkaloids is filtered off. Excess of magnesia is added, and the liquid evaporated to dryness, and the dry residue is extracted with chloroform: the chloroform is evaporated and the theobromine (and caffeine) weighed.

A full examination of numerous methods suggested for this determination has been made by Kunze ("Zeit. J. Analyt. Chem." 1894, 1). Kunze prefers the following process, which, however, is more tedious than that first described and but little more accurate. Ten grms. of cocoa are boiled with 150 c.c. of 5 per cent sulphuric acid for twenty minutes and the liquid filtered and the residue washed with boiling water.

Phosphomolybdic acid is then added to the liquid, and after standing for twenty-four hours, the precipitate is filtered and washed with 5 per cent sulphuric acid. While still wet, the precipitate is transferred to a flask and decomposed with baryta water. CO_2 is then passed through the liquid and the whole is evaporated to dryness. The dry mass is exhausted with hot chloroform, the chloroform evaporated and the dry residue, consisting of theobromine and caffeine, is weighed. Either of these processes gives quite satisfactory results.

Cold Water Extract.—Two grms. are placed in 100 c.c. flask and about 60 c.c. of cold water added. The whole is shaken well at intervals for several hours and then made up to 100 c.c., well shaken and allowed to stand all night. After again well shaking, 25 c.c. are filtered off, evaporated, and the residue weighed. Good commercial cocoa containing its full amount of fat contains on an average 12 to 13 per cent of cold water soluble extractive. In defatted cocoas this will be proportionally higher.

Determination of Pentosans.—Bodies known as pentosans are anhydrides of five carbon glucoses, which yield bodies of the type of xylose and arabinose on hydrolysis. According to Tollens these bodies appear to occur in greater quantity as lignification of plant substance progresses. Cross and Bevan consider that bodies of the oxycellulose type behave in this determination in the same way as pentosans. At all events, on distillation with hydrochloric acid such bodies yield furfural, from which the "pentosans" can be calculated. The method of carrying out this determination is as follows:—

Three to four grms. of the substance are mixed with 100 c.c. of hydrochloric acid of specific gravity 1.06 (= 12 per cent HCl) in a Wurtz flask and the contents of the flask distilled from a sand bath. When 30 c.c. has collected a further 30 c.c. of acid is added to the flask through a tap funnel and this is repeated till 400 c.c. is distilled over. During the distillation, a drop of the distillate is tested for furfural by touching it on paper impregnated with a dilute solution of aniline acetate with some sodium acetate. If no pink coloration appears, no more furfural is coming over and the distillation may be stopped. Usually between 300 to 400 c.c. will be distilled. A solution of phloroglucinol in dilute hydrochloric acid is then added to the distillate and the mixture is allowed to stand over night. The black precipitate formed is filtered through a weighed paper, washed with 150 c.c. of cold water, dried in a water oven and weighed. The weight of phloroglucide, divided by 1.82, gives the amount of furfural. This latter may be calculated into pentosans by subtracting 0.0104 gm. and multiplying by 1.88. The following results are given by Hehner and Skertchly ("Analyst," xxiv. 181), and refer to partially defatted cocoas.

COCOAS.

Cold Water Extract.	Nitrogen.	Fat.	Ash.	Alkalinity as K_2CO_3 .	Pentosans.
	Per cent	Per cent	Per cent	Per cent	Per cent
18.60	3.23	28.82	8.18	4.15	2.18
18.08	3.15	29.74	8.12	4.08	2.31
18.56	3.06	28.57	8.38	3.79	2.35
18.08	3.29	28.24	9.03	3.79	1.69
18.08	3.20	28.21	8.84	3.75	1.84
18.48	3.24	27.51	9.30	4.08	1.89
19.00	3.32	28.19	8.61	4.08	2.08
17.44	3.07	26.82	7.18	2.77	2.81

None of these samples show indication of the addition of husk.

CHOCOLATES.

Cold Water Extract.	Nitrogen.	Fat.	Ash.	Pentosans.	Fibre.
Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
67.20	0.76	23.76	2.17	1.27	—
66.88	0.80	23.12	1.98	0.83	1.18
65.04	0.70	23.59	2.16	0.81	1.33
42.96	0.60	4.20	—	1.88	2.95
37.52	0.57	4.21	—	1.81	2.71

The last two samples indicate the presence of about 25 per cent of "cocoa" which was almost entirely husks, as shown by the high fibre and pentosans. The determination of pentosans, however, gives little information that the estimation of fibre does not, and is far more complicated.

Added Alkali.—If a cocoa has an alkaline reaction, and yields an ash from 10 grms. which requires more than 1.5 c.c. of normal acid for neutralization, and the amount of ash insoluble in water is less than 50 per cent of the total ash, there is no doubt that a soluble alkali has been added to the cocoa. If the alkalinity is as high as above stated, and the insoluble ash is more than 60 per cent of the total ash, then magnesium carbonate has probably been added to the cocoa.

Microscopic Characters.—To examine powdered cocoa, two preparations should be made: (1) By thoroughly mixing a few grains with water; (2) By mixing with a little ether for a few hours, washing with alcohol and mounting in water. The tissues are now clearer than when mounted with water before extraction. The principal diagnostic characters of genuine powdered cocoa are as follow:—

- (1) Thin-walled parenchyma of the cotyledons.
- (2) Minute starch grains.

(3) Polygonal epidermis of the cotyledons with red-brown contents.

(4) Abundance of oil globules.

(5) Cells containing cocoa-red.

(6) Note the absence of large starch grains.

The principal portion of the cotyledons consists of polygonal thin-walled parenchyma, many of the cells containing minute starch grains and fat and albuminous matter. The starch grains are *round* and not

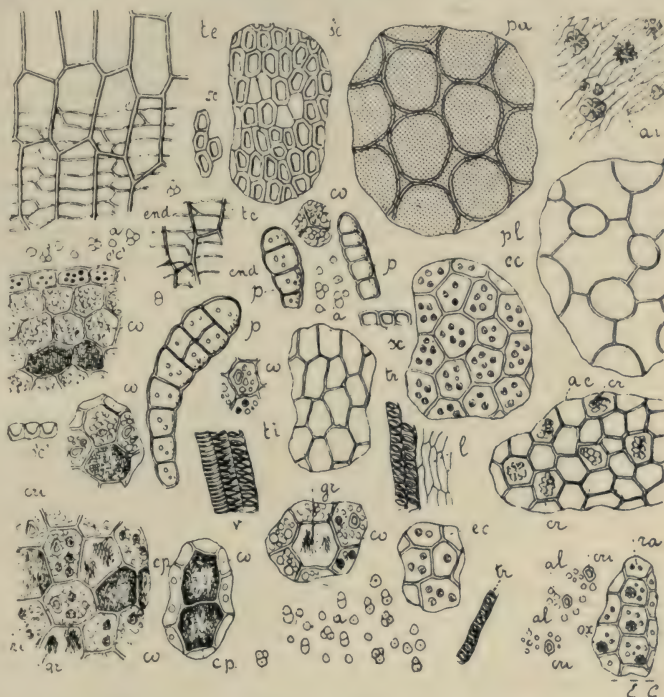


FIG. 1.—Powdered Cocoa. *a*, starch grains; *ae*, outer layer of endosperm; *ai*, inner layer of endosperm; *al*, aleurone grains; *co*, cotyledon; *cp*, pigment cells containing cocoa-red; *cr*, crystals of fat; *ec*, epidermis of cotyledon, surface view; *e'c*, epidermis of cotyledon, profile; *end*, inner epidermis of pericarp; *gr*, crystals of fat; *l*, bast from fibro-vascular bundles; *or*, calcium oxalate crystals; *p*, pluricellular hairs; *pa*, *pl*, parenchyma of seed coat; *ra*, cells of radicle; *sc*, sclerenchymatous layer of seed coat, surface view; *s'c*, sclerenchymatous layer of seed coat, in profile; *te*, outer epidermis of seed coat to which the inner epidermis of the pericarp (*end*) is adhering; *ti*, inner epidermis of seed coat; *tr*, *v*, vessels, etc., from fibrovascular bundle.

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angular. Attached to the epidermal cells, a few club-shaped hairs may be found. Characteristics of the husk are a large-celled epidermis and small thick-walled cells; and also spiral vessels of a well-

defined type, and masses of mucilage, which are tinged rose pink with ruthenium red dissolved in a 10 per cent lead acetate solution.

The diagnostic characters of powdered cocoa shells are as follows (Greenish):—

(1) The two epidermal layers: long narrow cells crossing larger polygonal ones, often diagonally.

(2) The small polygonal thick-walled cells of the sclerenchymatous layer.

(3) The large rounded parenchymatous cells with arm-like projections.

(4) The mucilage in masses, often tinged with brown.

(5) Spiral vessels or their fragments.

COCOA BUTTER.

It will now be convenient to briefly consider the principal characteristics of cocoa butter—the fat of the cocoa seed. This fat is used in medicine—principally for the preparation of suppositories—and is a drug official in the British Pharmacopœia—although it is principally used as a food, in the preparation of chocolate creams, etc. The official requirements of the Pharmacopœia are as follows: It softens at 26·6° C., and melts between 31·1° and 33·9°. If 1 gm. be dissolved in 3 c.c. of ether in a test tube at 17° C., and the tube be placed in water at 0°, the liquid should not become turbid, nor deposit in less than three minutes, and if the mixture after congealing be exposed to a temperature of 15·5° it should gradually afford a clear solution. This test is highly unsatisfactory. The following may be taken as the average character of genuine cocoa butter:—

Specific gravity ¹ at 15° C.	=	0·955 to	0·995
" " at 100°	=	0·855 "	0·858
" " at 15°	=	0·855 "	0·858
Melting-point		28° "	33° (but variable if the fat has been heated much).
Melting-points of fatty acids		48° "	51°
Reichert-Meissl value (5 grms.)		0·2 "	0·6
Saponification value		192 "	196
Iodine value		32 "	38
Refractive index at 40°	=	1·4565 "	1·4575
" " " at 60°	=	1·4496 "	1·4504
Butyro-refractometer index at 40°	=	46 "	48
" " " at 35°	=	48·5 "	49·5

Cocoa butter consists chiefly of the glycerides of stearic, lauric, palmitic, arachidic and oleic acids (with an acid of the empirical formula $C_{64}H_{128}O_2$ termed by Kingzett theobromic acid). A little cholesterin and some glycerides of the volatile fatty acids are also present. In determining the melting-point of this fat the capillary tube should be allowed to stand for forty-eight hours after the fat is first melted, before

¹ The specific gravity of cocoa butter gradually rises from about 0·950 after being freshly melted to a maximum of 0·995 after a few days. This figure should therefore be determined at $\frac{100^\circ}{15^\circ}$, at which temperature this disturbing influence is eliminated.

the determination is made. Or it may be stood in ice for six hours or so.

Adulteration.—It is stated in textbooks to be frequently adulterated with tallow, various fatty oils, paraffin wax and beeswax. This is quite untrue. Cocoa butter is rarely adulterated. The author has examined a very large number of samples and adulteration is rare—and when practised, not with, for example, beeswax, which is usually worth at least 25 per cent more than cocoa butter. Substitution—usually quite openly—of another fat is, however, common, and several so-called “cocoa butter substitutes” are on the market. The basis of these is either the stearin of cocoa-nut oil (sometimes from palm-nut oil)—which melts at a very low temperature and is a poor substitute for cocoa butter—or one of the harder and less known fats such as Shea butter.

The author has examined a number of these samples and found the following average figures:—

SUBSTITUTES WITH COCOA-NUT STEARIN AS THE PRINCIPAL INGREDIENT.

Specific gravity at $\frac{100^\circ}{15^\circ}$	0.8736	
Reichert-Meissl value (5 grms.)	4.5	to 6.0
Iodine value	4	„ 8
Saponification value	250	„ 270
Melting-point	26°	„ 28°
Refractive index at 60°	1.4400	„ 1.4420
Butyro-refractometer No. at 60°	33	„ 37

SUBSTITUTES OF THE SHEA BUTTER TYPE.

Specific gravity at $\frac{100^\circ}{15^\circ}$	0.855 to 0.865	
Reichert-Meissl value (5 grms.)	below 1	
Iodine value	30	to 45
Saponification value	180	„ 195
Melting-point	28°	„ 34°

SUBSTITUTES OF THE PALM-NUT STEARIN TYPE.

Specific gravity at $\frac{100^\circ}{15.5^\circ}$	0.873 to 0.875	
Melting-point	25°	„ 30°
Iodine value	10	„ 15
Saponification value	240	„ 255
Reichert-Meissl value (5 grms.)	5	„ 7
Refractive index at 60°	1.4430	„ 1.4450
Butyro-refractometer No. at 40°	36	„ 37

CHOCOLATE.

Chocolate is universally understood to be sweetened and flavoured cocoa. There is no legal standard for chocolate, and if one should ever arise, it would obviously be impossible to fix the relative proportions of the sugar and cocoa except within very wide limits. Many analysts are strongly in favour of fixing legal standards for this article, but in doing so grave difficulties would arise. The bulk of the chocolate manufactured is sold as a sweetmeat, and not for ordinary nutritive

purposes. It may be bought at very high prices, or by the poorer classes at so little as three ounces for a penny in the form of chocolate cream. The whole question becomes one of palatability, except in the case of chocolate sold as a beverage, and an obvious hardship would ensue if the sale of the lower grade, but quite wholesome, chocolate and chocolate creams were to be interfered with, because they contain, for example, the outer husk of the cocoa bean, or some cocoa-nut fat as a filling. It appears to stand on quite a different footing to a purely natural product where an obvious standard of quality exists. To restrict the sale of this product would logically necessitate carrying the principle to many other articles, and would largely interfere with the sale of inferior but still wholesome products. In the case of chocolate for use as a beverage it may be that standards would be advisable. Where nothing but cocoa husk is used, the term chocolate certainly ought to be qualified in some way.

The quantity of sugar in the best chocolate averages about 50 per cent, the remainder being pure cocoa either with or without the addition of some extra quantity of cocoa butter, with a minute quantity of flavouring. The principal flavour used to be derived from vanilla beans, and much of it is still obtained from that source, but the bulk of the vanilla flavour is now derived from synthetic vanillin obtained from oil of cloves. A little cinnamon, benzoin, Tolu and Peru balsams, and nutmegs are sometimes used, but vanilla is the favourite flavouring employed all over the world.

The fact that chocolate is only sweetened cocoa (for in the analysis the determination of the amount of flavouring is an impossibility in nearly all cases) renders a long description of its analysis unnecessary.

The points to which attention should be paid are as follows (1) Presence of husk, (2) The quantity of sugar, (3) The presence of foreign starch, (4) The addition of fats other than cocoa butter, especially common in the white portion of chocolate creams, and also in cheaper bar chocolate, and in the coverings of chocolate creams. The determination should include the following:—

Moisture—Mineral Matter.—If nothing but sugar and flavouring have been added, the ash will be a direct indication of the amount of cocoa present, especially if the microscopic examination shows that husk is absent. Rarely, a little iron earth is added to improve the colour of poor chocolate. This will give a higher ash in which iron can be detected. The water-soluble ash is, in genuine cocoa, about 50 per cent of the total; if it be higher than this, the presence of husk (or alkali) is probable. The siliceous matter of the ash as determined by the evaporation of the ash with HCl, and weighing the then insoluble residue is important, as it rarely exceeds 0.05 per cent in the cocoa nib, but reaches 1.2 per cent in the husk. The fat will be examined in the same way as with cocoa, the fibre also determined as described above, as well as the nitrogen (if required), the sugar and starch. The cold water extract of cocoa being tolerably constant (on the fat-free cocoa averaging 24 to 25 per cent), this figure which, in the case of chocolate will represent the cold water extract of the cocoa present, to-

gether with the sugar added, allows the amount of cocoa to be calculated. In estimating the value of the determinations on a sample of chocolate, corrections are to be made for the amount of added sugar found, when the results can be directly compared with cocoa standards. For the composition of a number of samples of chocolate reference should be made to the "Analyst" (xxxiv. 134).

Milk chocolate is a mixture of cocoa, sugar, milk-powder and various flavourings. According to Dubois ("Jour. Amer. Chem. Soc." 1907, 556) the following analyses represent typical milk chocolate of well-known brands:—

	Polarization.		Sucrose.	Lactose.	Per cent of milk fat in total fat.
	Direct.	After inversion at 24°			
			Per cent	Per cent	
1	+21°	-2°	40.9	8.24	22.1
2	+23.22°	-2.22°	45.7	9.12	22.9
3	+23.88°	-2.20°	46.8	8.24	24.2

When both sucrose and lactose have to be determined, as in the case of a milk chocolate Dubois' method may be used. Thirteen grms. of the sample are freed from fat, and to the residue 100 c.c. of water is added and the whole well shaken for ten minutes, 5 c.c. of basic lead acetate solution are then added, and the solution filtered, and excess of lead removed by H_2S ; 25 c.c. of the filtrate are allowed to stand overnight in order to attain its stable rotation and the polarimetric value determined. Multiply this reading by 2. 50 c.c. of the filtrate are inverted by acid, neutralized and made up to 100 c.c. Take the polarimetric reading at the same temperature as the direct reading and also take the reading at 86°. Multiply the readings by 2. The weights of the two sugars may be calculated from the following formulæ:—

$$\text{Sucrose (in grms.)} = \frac{(a-b) 1.05}{142.66 - \frac{t}{2}} \times 13$$

$$\text{Lactose} = \frac{19.152 c}{100}$$

When a is the direct reading for normal weight.

When b is the invert reading for normal weight.

When c is the invert reading at 86°.

Baier and Neumann ("Analyst," xxxiv. 439) give the following details for the examination of milk or cream chocolate. The quantity of milk-fat and casein present should be estimated; the percentage of milk-fat can be obtained from the Reichert-Meissl value of the fat separated from the chocolate, whilst the amount of casein can be ascertained by a modification of Hammarsten's method, which relies on

the solubility of casein in ammonium oxalate solution and on the insolubility of other proteins in this solution. The authors have found that casein is completely soluble in sodium oxalate, but not in ammonium oxalate. To estimate casein proceed as follows: Extract 20 grms. of the powdered chocolate with ether in a Soxhlet apparatus for sixteen hours; allow the extracted residue to dry spontaneously, and rub down 10 grms. of it in a mortar with a small quantity of 1 per cent sodium oxalate solution. Wash the paste into a 250 c.c. flask with about 200 c.c. of the oxalate solution, heat the mixture to boiling and add hot sodium oxalate solution until the flask is nearly filled up to the mark. Allow it to stand for about eighteen hours, occasionally shaking, then dilute with cold sodium oxalate solution to a volume of exactly 250 c.c.; mix and filter. Add 5 c.c. of 5 per cent uranium acetate solution to 100 c.c. of the filtrate, then 30 per cent acetic acid drop by drop, continually stirring until the casein commences to precipitate. The number of drops necessary vary from 30 to 120 according to the quantity of casein present. Add 5 drops of the acetic acid in excess, separate the precipitate by centrifugal action, and wash with a solution containing 5 grms. of uranium acetate and 3 c.c. of 30 per cent acetic acid per 100 c.c. As soon as the washings give no reaction for oxalates, transfer the precipitate to a flask and determine the quantity of nitrogen present by Kjeldahl's process. Multiply this quantity of nitrogen by 6.37, thus obtaining the amount of casein, calculating it into a percentage quantity on the original chocolate. When calculating the percentage of milk-fat it is assumed that cocoa butter has a Reichert-Meissl value of 1.0 and milk-fat a value of 27.0. The total milk solids present in the chocolate can be estimated from the quantities of casein and fats therein. Fat, proteins, lactose and mineral matter are the component parts of the milk solids. To calculate the proteins multiply the casein by 1.111; the lactose, multiply the protein by 1.3, and the mineral matter, multiply the protein by 0.21. The fat content of the milk or cream used in the preparation of the chocolate can be estimated by a simple calculation. The authors state that they consider that the milk used should contain about 3.5 per cent of fat, and the cream about 10 per cent. Milk chocolate should in their opinion contain at least 15 per cent of dry milk solids, while cream chocolate should contain at least 20 per cent of dry cream solids.

COFFEE.

Coffee berries are the seeds of *Coffea Arabica* and probably of allied species of the natural order *Cinchonaceae*. The commercial product consists of the endosperm of the seed, the seed coats having been removed during the preparation of the coffee. Small fragments of the seed coats, however, may be found in the groove running along the flat side of the berry, and naturally, also in ground coffee. The coffee tree is cultivated in many tropical countries, into which it has been introduced from Abyssinia and Ethiopia. India, Java, Ceylon and Arabia furnish some of the best coffee, but at least half of the world's supply comes from Brazil. The quality of coffee is a matter for the

palate, and not for chemical analysis, the function of which is merely to decide on its purity.

The raw coffee berries are roasted to a greater or less degree before use, and the greater part of the coffee sold in commerce consists either of the roasted beans, as the berries are usually termed, or of the same ground to a coarse powder. It is during the roasting of the berry that the peculiar aroma of coffee is developed, and the original toughness of the berry is destroyed, so that it can then be easily ground. The changes brought about by roasting are as follows: A large quantity—up to 20 per cent—of water and organic matter is driven off and the sugar present is largely caramelized: a small amount of caffeine is probably volatilized, but not very much. The fat and albumen are partially decomposed, carbonic acid gas is given off and the berries naturally swell. Traces of quinone, acetone, methylamine and similar bodies are formed, and also a certain amount of caffeol, $C_8H_{10}O_2$, which is a heavy oil which appears to be responsible for the aroma of roasted coffee. Samples of raw coffee, roasted in the author's laboratories to a full rich brown colour, gave the following results, indicating the changes which take place during roasting:—

	I.		II.	
	Raw.	Roasted.	Raw.	Roasted.
	Per cent	Per cent	Per cent	Per cent
Moisture	12.45	4.1	11.9	3.7
Ash	3.72	3.95	3.66	3.82
Cellulose	26.82	25.00	28.5	26.8
Caffeine	1.2	1.36	1.36	1.40
Sugar	4	1.5	3.2	1.1

The Constituents of Coffee.—The principal constituents of coffee are (1) Caffeine (identical with theine), (2) Caffetannic acid, $C_{15}H_{18}O_8$, in combination with caffeine and with magnesia or lime, (3) Fat, (4) Albumenoids, (5) Carbohydrates, (6) Essential oil and aromatic substances, (7) possibly other alkaloidal substances than caffeine.

Adulterants of Coffee.—Coffee is subject to a considerable amount of adulteration; this is generally only to be found in the ground variety, although numerous cases of factitious coffee beans have been noticed, but these latter are usually easily detected by the eye by any skilled observer. The principal adulterant met with to-day is roasted chicory, the root of *Cichorium Intybus*.

Roasted rye, wheat, dates, acorns and other similar vegetable matter have occasionally been found, but to-day these are rarely used. Sometimes, according to König, the berries are roasted with glucose, which provides much caramel and makes the resulting infusion appear stronger. Exhausted coffee, which has been used for the manufacture of extract of coffee, is also a recorded, but rare, adulterant. The

factitious beans are moulded from one or more of the following ingredients; chicory and other roasted vegetable matter, china clay, wheat flour, bran, sawdust, caramelized sugar, and lupin seeds. But, as

Substance.	Ash.	Fat.	Sugar.		Water.	Soluble in H ₂ O.	Observers.
			Original.	After inversion.			
Coffee	Per cent 4 to 6.5	Per cent 11 to 16	Per cent 0.2	Per cent 22 to 25	Per cent 1 to 4	Per cent 21 to 26	Parry
"	4.2 " 6.4	11.8 " 15.6	0.2	24.3	1.5 " 4.5	22.5 " 25.2	Krauth
"	3.5	14.35	0.7	—	1.29	—	Rupp
Chicory (roasted)	9 " 12	1	22.24	22.25	2 " 4	62 to 67	Parry
"	10.8	1.15	23.4	22.14	4.3	65.4	Krauth
Acorns (roasted)	2.12	4.61	8.05	—	12.85	—	König
Wheat (roasted)	1.8	2.75	—	—	—	52 to 65	Krauth

mentioned above, the adulterant found in the vast majority of cases is roasted chicory root.

Riley gives the following list of imitation "coffees" which have been found in the course of examinations made in the laboratory of the

United States Department of Agriculture. The mixtures had been moulded and pressed into berries:—

Coffee, bran and molasses.

Wheat-flour, coffee and chicory.

Wheat-flour, bran and rye.

Chicory, peas and barley.

Wheat, oats and buckwheat.

Wheat-flour and sawdust.

Husks of leguminous seeds roasted, and molasses.

Pea hulls and bran.

Factitious coffee berries, however, are rarely met with to-day.

The analyses on page 32 represent the principal constituents of coffee and several of the adulterants noted above.

Dyer ("Analyst," XXIII. 226) gives the following table of figures for dried chicory (the moisture varied from 1 to 4 per cent):—

	Insoluble in water	Ether extract.	Nitrogen.	Ash.	Soluble Ash.	San l
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Chicory nibs, medium roast	22.40	2.57	1.53	4.63	2.50	0.70
" " dark roast	50.30	1.43	1.67	4.70	2.99	0.30
Ground chicory	22.27	2.17	1.33	5.53	2.43	1.43
"	21.50	1.90	1.34	5.23	2.07	1.43
"	35.50	3.43	1.50	5.13	2.57	0.77
"	37.80	3.87	1.52	8.23	1.60	3.97
"	22.77	3.17	1.25	5.13	3.30	1.66
"	22.50	3.67	1.23	5.73	3.23	1.63
"	23.50	2.60	1.29	5.63	2.97	1.47
"	22.50	2.60	1.29	5.33	3.22	1.47
"	22.63	2.57	1.29	5.70	2.80	1.47

The following figures are given by Allen as representing certain factitious "coffees":—

	"Acorn Coffee."	"Rye Coffee."	"Barley Coffee."	"Barley Coffee."
	Per cent	Per cent	Per cent	Per cent
Water	12.85	2.22	3.45	6.41
Nitrogenous matter	6.13	11.87	9.38	10.56
Fat	4.01	3.91	3.25	1.04
Sugar	8.01	—	—	—
Starch	—	8.34	—	—
Dextrin	62.00	49.51	70.13	68.38
Other non-nitrogenous matter	—	9.83	—	—
Cellulose	4.98	9.78	4.25	10.50
Ash	2.02	4.54	3.36	3.04
Soluble in H ₂ O	—	61.33	31.20	34.37
Glucose (by inversion)	—	—	69.28	67.19

Tatlock and Thomson ("Jour. Soc. Chem. Ind." 1910, **29**, 138) give the following results of the analyses of a number of coffees, of a coffee "free from caffeine," and a sample of chicory :—

	Caffeine.	Water Extract.	Ash Soluble in H ₂ O.	Ash Insoluble in H ₂ O, less SiO ₂ .	Silica.	Specific gravity of 10 per cent Infusion.
	Per cent	Per cent	Per cent	Per cent		Per cent
Costa Rica	1.22	30.80	3.06	0.77	trace	—
"	1.20	30.26	2.96	0.88	"	1.0102
"	1.38	30.77	3.21	0.98	"	1.0099
Mysore	1.18	31.02	3.01	0.93	"	—
"	1.25	29.06	3.15	0.96	"	1.0102
E. India	1.46	29.10	3.32	0.97	"	1.0101
Mocha	1.19	30.76	3.14	0.85	"	1.0102
Caffeine-free	0.08	27.42	3.30	1.01	"	1.0101
Chicory	none	75.84	1.95	2.01	4.77	1.0274

These chemists consider that 30 per cent of matter extracted by water for coffee and 75 per cent for chicory (calculated on the dried substance) affords a fair basis for calculation of mixtures of the two.

THE ANALYSIS OF COFFEE.

Specific Gravity.—Much stress has been laid on the specific gravity of coffee by the chemists of the Municipal Laboratory of Paris. By the use of elaborate apparatus they have determined this figure for a number of samples, and give 1.041 to 1.368 for unroasted, and 0.500 to 0.635 for roasted coffee. The author has found that for ordinary cases this figure yields absolutely no information. It is of use only in a few cases where actual factitious whole beans are present, for these are almost invariably heavier than water, whilst genuine roasted beans are lighter—unless, according to Allen, they have been much over-roasted. Allen takes the average specific gravity, if the beans do not float in water, and are therefore presumably factitious, by immersing twenty beans in brine and gradually adding water until ten sink and ten float. The specific gravity of the liquid is taken as the mean specific gravity. This determination is certainly of but little value and does not justify the amount of trouble that has been taken over it.

The following determinations should be made :—

Ash.—The ash of pure coffee is usually between 3.5 and 4.5 per cent, rarely exceeding 4.8 per cent. The ash of chicory is distinctly higher, and this figure may afford useful information on analysis. The composition of the ash of coffee, however, differs in a marked degree from that of chicory, and by determining the amount of soluble ash and silica present an approximation as to the amount of chicory present may be made. Not less than 60 per cent—usually 70 to 80 per cent of the ash of pure coffee—is soluble in water, whereas only 25 to 35 per cent of the ash of chicory is soluble in water. The ash of coffee is free from silica, whereas that of chicory contains a not-

able quantity. The following figures may be taken as covering most samples:—

	Coffee.	Chicory.
	Per cent	Per cent
Silica and Sand	—	10.69 to 55
CO ₂	14.92	1.78 „ 3.19
Fe ₂ O ₃	0.44 to 0.98	3.13 „ 5.32
Cl	0.26 „ 1.11	3.28 „ 4.93
P ₂ O ₅	10 „ 11	5 „ 6
Soluble Ash	75 „ 85	21 „ 35

It is evident, therefore, that an analysis of the ash renders very useful information.

A comparison, however, of the soluble ash in the two substances is often vitiated by the high amount of sand present in the chicory root. As Allen points out, by comparing the soluble ash with the total ash minus the sand and silica, more reliable results are obtained, and a rough indication of the percentage of chicory present may be deduced. With pure coffee, the amount of soluble ash is, as given above, usually 75 to 85 per cent, whereas in chicory (after deducting the sand and silica) it is from 38 to 45 per cent or thereabouts.

The ash of dandelion root also contains a high amount of silica—varying from 10 to 14 per cent of the total ash.

Determination of Fat.—The fat of pure coffee is fairly constant in amount. It may be determined in the dry powdered coffee by extraction with ether and petroleum ether and varies between 10 and 14 per cent, or in rare cases 16 per cent. Chicory yields about 1 to 2 per cent, so that any undue proportion of this substance will be indicated by the low fat yield. Most other adulterants yield a very low fat value.

Aqueous Extractive.—The watery extract of coffee is not only very constant in amount, but is sensibly less than that of chicory. Instead of actually weighing the extracted matter, Graham, Stenhouse and Campbell ("Journal Chem. Soc." ix. 38) preferred to take the specific gravity of the aqueous infusion. Their method was to take the powdered substance with ten times its weight of cold water and raise the liquid to the boiling-point, and on cooling to 15.5° to take the specific gravity. The following were the results obtained:—

Substance.	Specific gravity.	
	Per cent	Per cent
Coffee	1.008 to 1.009	Average 1.0087
Chicory	1.0191 „ 1.0233	„ 1.02105
Leguminous seeds	1.0057 „ 1.0084	
Acorns	1.0073	
Dandelion root	1.0219	
Cereals	1.0109 „ 1.0263	

Allen, by well boiling the coffee and filtering and washing till the filtrate measured 10 c.c. for each gramme of the coffee employed, found 1.0079 as the average specific gravity. He prefers to dry the sample first, and then adopts the values 1.009 for coffee and 1.024 for chicory. On this basis which yields fairly approximate results, the percentage of coffee in a mixture of chicory and coffee may be approximately deduced from the equation,

$$P = \frac{(1024 - d) 100}{15}$$

where P is the percentage of coffee, and *d* is the specific gravity of the 10 per cent infusion.

McGill prefers to boil the finely powdered sample for an hour under a reflux condenser and filter and make up to the requisite volume. He then adopts the values 1.00986 for coffee and 1.02821 for chicory.

Macfarlane, after extracting the dried sample with petroleum ether, and again drying the sample, then extracts with water. He gives the following table for the dried watery extract obtained:—

	Per cent
Soutas coffee	22.44
Mocha "	21.92
Java "	20.42
" and 10 per cent chicory	25.90
" " 20 " " " "	30.75
" " 30 " " " "	37.40
" " 40 " " " "	43.36
" " 50 " " " "	49.84
" " 60 " " " "	53.82
" " 70 " " " "	60.34
" " 80 " " " "	65.93
" " 90 " " " "	71.41
Chicory	77.73

Calculated on the dried substance 24 per cent may be taken as the value for the aqueous extract of normal coffee and 75 per cent for that of chicory. These are fair average values.

As the results of the examination of over 100 samples the author prefers to adopt Allen's suggestions—namely, of boiling the sample for twenty minutes, with about six times its weight of water, filtering and washing with warm water and making up to ten times the weight of the coffee used at 60°. The values 1.009 for coffee and 1.025 for chicory will then give very close results. The equation $P = \frac{(1025 - d) 100}{16}$

will then give the amount of coffee in a mixture, where P is the percentage and *d* is the specific gravity.

A. E. Johnson prefers to weigh the aqueous extract, which he gives as 24 per cent for *dried* roasted coffee, and about 70 per cent for dried chicory. (Hegner has found as low as 54.1 per cent in a pure chicory.) To determine the extractive matter Johnson boils 5 grms. with 200 c.c. of water for 15 minutes, strains, and again boils the residue with 50 c.c. of water for 5 minutes. The liquids are mixed, made up to 250 c.c.

when cold, and filtered; 50 c.c., equivalent to 1 grm. of the sample, are dried over a water bath and finally in a water oven, and weighed. The amount of coffee in the sample is calculated on the average values 24 and 70 as mentioned above. This method is simple and gives excellent results.

The following are average values for the water-soluble extractives in certain substances used as adulterants of coffee:—

Roasted rye	30 to 36.5 per cent
Roasted wheat	47 „ 55 „
Roasted figs	60 „ 67.5 „

The refractive index of the aqueous infusion (10 per cent) has been used as a means of discriminating between genuine coffee and some of its substitutes.

Lythgoe has examined a number of samples and finds the refractive index of the 10 per cent extract at 20° to lie between 1.3374 and 1.33804.

The same extract of roasted chicory had a refractive index 1.34463.

Graham, Stenhouse and Campbell (*"Journ. Chem. Soc."* ix. 36) suggested a comparison of the tinctorial value of coffee infusions as a method of detecting and determining the amount of adulterants. They found that infusions of pure coffee, compared with similar infusions of chicory in the usual manner in Nessler glasses, showed only one-third of the colour of the latter. Allen considers that 2.8 to 3.2 was a fair value for the colour of the chicory infusion as compared with that of coffee and recommends a standard colour being kept which is made up of ferric, cobalt, and copper sulphate, of exactly the depth of tint as a standard 1 in 200 infusion of 50 per cent coffee and 50 per cent chicory. Such a standard is unalterable and can be kept. An infusion of the sample to be compared is made (1 in 100) and if it be pure coffee, the colour will be equal to that of an equal volume of the standard colour. If chicory be present, the colour will be darker, and water must be added until the two tints are identical. This is done in graduated Nessler tubes and the amount of chicory present is calculated on the basis of the figures 3 for chicory and 1 for coffee.

The author has examined this tinctorial comparison with samples of coffee and chicory which have been roasted for different lengths of time under different conditions, and consider that very variable results may be obtained from the same sample thus differently treated.

It is considered a waste of time to make the comparison when so much more reliable results can be obtained by so simple a method as the determination of the specific gravity of the aqueous extract.

Determination of Caffetannic Acid.—Digest 2 grms. of the powdered sample for thirty-six hours with 10 c.c. of water: then add 25 c.c. of 90 per cent alcohol and continue the digestion for twenty-four hours. The liquid is then filtered and the residue washed with 90 per cent alcohol. The filtrate is heated and a boiling concentrated solution of lead acetate is added, which causes the precipitation of caffetannate of lead, which contains 49 per cent of lead. When this has become flocculent, it is filtered off, and washed with 90 per cent alcohol, until the washings are free from lead, and then with ether until free from fat, dried at

100° and weighed. The precipitate may be taken as containing 50 per cent of caffetannic acid.

Determination of Pentosans.—(See under cocoa, p. 23.) Hehner and Skertchly give the following results for some coffees and chicories:—

	Moisture.	Pentosans.	Crude Fibre.
	Per cent	Per cent	Per cent
Raw coffee	—	2.86	—
Roasted „	—	2.50	7.36
Coffee with 32 per cent chicory	—	2.71	—
High dried Belgian chicory	5.51	5.14	5.47
„ „ roasted 5 minutes	6.17	5.55	6.57
„ „ „ 10 „	3.95	5.16	6.87
„ „ „ 15 „	3.73	4.80	8.67
„ „ „ 23 „	3.28	5.56	11.50

(Carbonized matter is included with the fibre.)

The Determination of Caffeine.—This determination is not often required in samples of coffee, as the quality of coffee does not bear a direct ratio to its alkaloidal value.

Paul and Cownley ("Ph. Jour.," [3] xvii. 565, 648, 821, 921) recommend the determination as of value, as the amount of alkaloid is, they claim, fairly constant, and a determination may give an indication as to the amount of coffee in a mixture. They showed that most published processes gave results below the truth. They find in dried coffee 1.20 to 1.39 per cent of caffeine, and adopt 1.3 per cent as a mean value. Allen prefers the figure 1.2 per cent as a safer average. Paul and Cownley operate as follows:—

Five grms. of the sample, finely powdered, are well mixed in a mortar with 2 grms. of calcined magnesia, the whole moistened with hot water, well triturated and then dried at 100°. The mixture is extracted with boiling alcohol, and the resulting liquid evaporated nearly to dryness. It is then boiled with 50 c.c. of water and heated with a few drops of dilute H_2SO_4 . When cold, the liquid is repeatedly extracted with chloroform until exhausted. The mixed chloroform solution is then treated with a 1 per cent solution of caustic alkali to remove colouring matter, and the separated chloroform solution evaporated and the practically pure caffeine weighed.

According to Allen, a trace of caffeine is left in the sample by this treatment, and can be extracted by water *after* the alcoholic extraction. He also states that six or seven successive extractions with 30 to 40 c.c. of chloroform are necessary.

In case the resulting caffeine—as is often the case with caffeine extracted from coffee, but not so with caffeine from tea—should be somewhat brown (due to the presence of a waxy or resinous impurity), it should be purified by re-solution in boiling water and subsequent filtration, and evaporation of the water.

There are numerous other methods of determining the caffeine, but as they are usually slight variations one of the other, and give results

of, generally, less accuracy than the above, it is not necessary to more than refer the reader to the papers of Paul given above, that of Allen's pupils ("Pharm. Journ." [3], xxiii. 215), and to briefly draw attention to the process preferred by Allen and those recommended by Juckenack and Hilger, and by Lendrich and Nottbohm.

Allen exhausts 12 grms. of the finely powdered coffee by boiling under a reflux condenser with 500 c.c. of water. After six to eight hours boiling, the liquid is filtered, and the residue washed on a filter, making the filtrate up to 600 c.c. This is then heated to about 95° C., and about 4 grms. of powdered lead acetate added, and the whole boiled under a reflux condenser for ten minutes. If the precipitate does not curdle and settle readily, leaving the liquid of at most a pale colour, a further addition of lead acetate, and further boiling are necessary. The liquid is now filtered, after it has been made up to 600 c.c. when cold, and 500 c.c. of the filtrate (equivalent to 10 grms. of coffee) are evaporated to about 50 c.c. and a little sodium phosphate is added to precipitate the remaining lead. The liquid is filtered, the precipitate washed, and the filtrate is further concentrated to about 40 c.c., when the caffeine is extracted in a separator by four to five successive treatments with chloroform. The chloroform solutions are mixed, the chloroform distilled off in a tared flask, over a water bath. The last traces of chloroform are removed by a current of air, and the alkaloid weighed.

In the presence of chicory the caffeine is liable to be strongly coloured, and it should then be redissolved in water, a few drops of solution of caustic alkali added, and the liquid again extracted as before with chloroform.

Juckenack and Hilger exhaust 20 grms. of the sample with 900 c.c. of water by boiling for about three hours, and whilst the liquid is cooling, add 75 c.c. of a 10 per cent solution of basic aluminium acetate when the thermometer marks about 70°, and then 2 grms. of sodium bicarbonate. The liquid is again boiled for five minutes, and on cooling made up to 1020 c.c., 750 c.c. of which is filtered. This is equivalent to 15 grms. of coffee. This liquid is evaporated with 10 grms. of powdered aluminium hydroxide and the residue is extracted with carbon tetrachloride in a Soxhlet tube. The solvent is evaporated and the alkaloid weighed. The residue should be slightly moistened with water before extraction—or all the caffeine will not be dissolved. [It is noted that in treating an aliquot part of the liquid this process allows for the 20 grms. of coffee added, whilst Allen makes no such allowance.]

Lendrich and Nottbohm ("Zeit. Untersuch. Nahr. Genuss." 1909, 17, 241) give the following as the most accurate method of obtaining the caffeine in a state of great purity. Twenty grms. of the coffee ground to a fine powder, are moistened with 10 c.c. of water, the mass being stirred from time to time for a period of two hours. The moist mass is then transferred to an extraction thimble and extracted with carbon tetrachloride for three hours. To the extract 1 gm. of paraffin wax is added, and the carbon tetrachloride is evaporated. The residue is extracted with four successive quantities of boiling water, using 50 c.c. for the first extraction and 25 c.c. for each of the subsequent ones.

The united extracts are passed through a moistened filter paper which is washed with hot water. The filtrate is treated with 10 to 30 c.c. of a 1 per cent solution of permanganate of potash for 15 minutes at ordinary temperature, and the excess of permanganate destroyed by the addition of a 3 per cent solution of hydrogen peroxide containing 1 per cent of acetic acid. The whole is then heated on a water bath and filtered, the residue being washed with hot water. The filtrate is now evaporated to dryness, the residue dried in a steam oven and at once extracted with warm chloroform. The chloroform is evaporated, and the resulting caffeine dried at 100° for thirty minutes and weighed.

Microscopic Examination.—An examination of powdered coffee under the microscope affords the principal means of identifying adulterants in coffee. In this examination it is useful to compare the tissues with those found in sections of the raw coffee beans, since, with the exception of some alteration in the cell contents, roasting has no material effect on the appearance of the tissues. To fully examine coffee, the fine and coarse particles may be separated by a sieve and sections of the coarser particles made. The tissues are too dark for useful observation, and may be decolorized by sodium hypochlorite and mounted in diluted glycerine. The principal portion of coffee is composed of fragments of the endosperm—thick-walled angular cells, very tough and hard, and adhering firmly to each other. Globules of oil are enclosed in the cells but no starch is present in them. The epidermal and immediately neighbouring cells have evenly thickened walls, but the remainder of the endosperm tissue consists of parenchymatous cells with thickened walls and very large pits—often as long as the cell is wide. A small portion of the seed coat (the bulk of which has been removed during the preparation of coffee for use) is always present, and is revealed by its very long sclerenchymatous cells, with numerous pits. A few spiral vessels are to be found—derived from the raphe or groove of the berry, but these are not numerous. The minute number of embryonic cells are rarely identifiable in ground coffee. These characters are sufficient to distinguish coffee from all its adulterants. The presence of the various starches is easily detected, and such adulterants as ground dates, roasted figs, etc., are widely different in their microscopic characters from coffee beans. The principal adulterant, chicory, reveals numerous loose, thin-walled parenchymatous cells, laticiferous vessels, sieve-tubes with transverse plates and large vessels with well-defined large pits, which cannot possibly be mistaken for ground coffee.

Roasted date stones, which have been used as a substitute, and probably also as an adulterant of coffee, have a very characteristic appearance under the microscope. The epidermal cells are almost oblong, whilst the parenchymatous tissue consists of very irregular-shaped cells containing much tannin.

CHAPTER II.

MILK, BUTTER, CHEESE, AND EDIBLE OILS.

MILK.

MILK is the fluid secreted by the mammary glands of female mammals for the nourishment of their young. It contains all the essentials of a complete food, namely, fat, sugar, proteids and mineral matter. The only milk of importance from the point of view of the present work is cow's milk.

Although milk is a natural product of variable quality, it is one of the few foods in regard to which section 4 of the Sale of Food and Drugs Act of 1899 empowers the Board of Agriculture to make legal standards. In pursuance of that power, the Board of Agriculture issued the following regulations on 5th August, 1901.

Milk.—(1) When a sample of milk (not being milk sold as skimmed, or separated, or condensed milk) contains less than 3 per cent of milk-fat, it shall be presumed for the purposes of the Sale of Food and Drugs Acts, 1875 to 1899, until the contrary be proved, that the milk is not genuine, by reason of the abstraction therefrom of milk-fat, or the addition thereto of water.

(2) When a sample of milk (not being milk sold as skimmed, or separated, or condensed milk) contains less than 8.5 per cent of milk solids other than milk-fat, it shall be presumed for the purposes of the Sale of Food and Drugs Acts, 1875 to 1899, until the contrary is proved, that the milk is not genuine, by reason of the abstraction therefrom of milk solids other than milk-fat, or the addition thereto of water.

Skimmed or Separated Milk.—(3) When a sample of skimmed or separated milk (not being condensed milk) contains less than 9 per cent of milk solids, it shall be presumed for the purposes of the Sale of Food and Drugs Acts, 1875 to 1899, until the contrary be proved, that it is not genuine, by reason of the abstraction therefrom of milk solids other than milk-fat, or the addition thereto of water.

(4) These regulations shall extend to Great Britain.

There are several important legal decisions in reference to this matter, which will be discussed in Volume II, but it may be well to here mention that milk below the standard is not necessarily to be regarded as adulterated, nor is milk above the standard necessarily to be

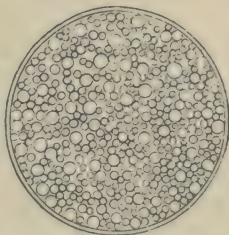


FIG. 2.—Microscopic appearance of milk.

regarded as pure. The effect of these regulations is to fix the burden of proof on the defendant where the milk was presumably adulterated ; in other words, the failure to satisfy the standards raises a presumption of adulteration, but one which is rebuttable by evidence.

Milk is essentially an emulsion of fine globules of fat in a solution containing milk sugar, casein and mineral salts. Under the microscope, the cream has the appearance shown on p. 41. The fat globules vary much in size, varying from about 0·0015 mm. to 0·009 mm. in diameter. Minute particles of separated proteids may sometimes be seen. Colostrum is the term applied to the milk yielded by the females for a few days after the birth of their young and differs materially in composition from normal milk, from which it should be absent. It may be recognized under the microscope by its containing numerous circular cells containing fat globules which have not yet been liberated by the disintegration of the cell wall.

Fresh cow's milk is usually amphoteric in reaction, that is, it yields both acid and alkaline reactions. To phenol-phthalein, however, it is always slightly acid. It soon becomes distinctly acid owing to a gradual conversion of lactose into lactic acid.

The Composition of Milk.—The qualitative composition of milk is fairly constant, but the quantities of the various constituents are liable to considerable variation. The following gives the average composition of normal cow's milk :—

Water	87·3 per cent.
Fat	3·6 "
Proteids	3·8 "
Lactose	4·5 "
Mineral matter	0·7 "
Citric acid	0·1 "
Colouring matter	traces

The fat of milk, which will be dealt with under butter, is a mixture of glycerides of the non-volatile fatty acids (olein, palmitin, stearin, and myristicin, about 90 per cent) and of the glycerides of the soluble volatile fatty acids (butyric, caprylic and caprinic, 10 per cent).

The proteid matters of milk are composed of the following substances : Casein constitutes about 80 per cent of the total proteids of milk and is probably only partly dissolved in the milk and partly held in a somewhat colloidal state in the liquid. It is a white, colourless, almost tasteless solid, soluble in alkalis, but precipitated by dilute acids. It is laevo-rotatory, alkaline solutions having a specific rotation of about -90° . Lactalbumin is the soluble albumin of milk and is present to the extent of about 15 per cent of the proteids. It resembles egg albumin, and is coagulated at 70 to 72° . It is readily soluble in water and has a specific rotation of about -68° . There are traces of a proteid which has been termed lactoglobulin, and numerous others have been described from time to time. They are, however, of little importance from an analytical point of view, and their literature may be found well summarized by Droop Richmond ("American Chem. Jour." 1893, October).

Lactose will be found fully described under "Carbohydrates".

The mineral matter of milk has the following average composition :—

	Per cent.
Potassium oxide	25.02
Sodium "	10.01
Calcium "	20.01
Magnesium "	2.42
Iron "	0.13
SO ₃	3.84
P ₂ O ₅	24.29
Chlorine	14.28

As the result of the examination of over 170,000 samples Vieth and Richmond give the following average values :—

Specific gravity at 15° C.	Total Solids.	Fat.	Solids not Fat.
Per cent	Per cent	Per cent	Per cent
1.03215	12.86	4.02	8.84

These analyses extended over fifteen years, the minimum and maximum average for any year being as follows :—

	Specific gravity at 15° C.	Total Solids.	Fat.	Solids not Fat.
	Per cent	Per cent	Per cent	Per cent
Minimum	1.0315	12.66	3.84	8.68
Maximum	1.0323	13.06	4.22	8.88

For the past ten years, the average fat value has been 3.75 per cent, according to Richmond.

Naturally there are variations outside these limits, for even milk of normal quality, and, naturally, still greater variations for milk obtained under abnormal conditions.

Milk cannot be considered normal unless it consists of the well-mixed total quantity obtained in a milking. The first portion of the milk leaving the udder is known as fore milk and contains less fat than the last portion, known as the strippings, which may contain up to 8 or 10 per cent of fat. The evening milk is nearly always richer in fat than the morning milk. The following represent average differences between fore milk and strippings on the one hand, and morning and evening milk on the other :—

	Water.	Total Solids.	Fat.
	Per cent	Per cent	Per cent
(a) Fore milk	88.0	12.0	1.4
Strippings	82.0	18.0	8.8
(b) Fore milk	88.5	11.5	1.45
Strippings	81.0	19.0	9.6
(c) Fore milk	87.8	12.2	1.25
Strippings	80.8	19.2	9.7

Morning Milk.

Sp. gr.	Solids.	Fat.
1.0322	12.53	3.63

Evening Milk.

Sp. gr.	Solids.	Fat.
1.0318	12.94	4.04

The variations in the composition of morning and evening milks throughout twelve months are fully recorded by H. D. Richmond ("Analyst," xxiv. 197).

The following analyses of colostrum are due to Engling:—

Time after Calving.	Specific gravity.	Fat.	Casein.	Albumin.	Sugar.	Ash.	Total Solids.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Immediately	1.068	3.54	2.65	15.56	3.0	1.18	26.93
10 hours	1.046	4.66	4.28	9.32	1.42	1.55	21.23
24 "	1.043	4.75	4.50	6.25	2.85	1.02	19.37
48 "	1.042	4.21	3.25	2.31	3.46	0.96	14.19
72 "	1.035	4.08	3.33	1.03	4.10	0.82	13.36

Milk from underfed cows comes within the category of abnormal milk. It has been carefully examined by Carter-Bell ("Analyst," vi. 63); he gives the following figures:—

Specific gravity.	Total Solids.	Fat.	Ash.
Per cent	Per cent	Per cent	Per cent
1.028 to 1.031	9.10 to 13.7	1.06 to 4.34	0.64 to 0.75

Milk that has been frozen is abnormal, since it is principally the water that freezes out. Droop Richmond ("Analyst," xviii. 53) gives the following figures:—

	Frozen Portion.	Unfrozen Portion.
	Per cent	Per cent
Water	96.23	85.62
Fat	1.23	4.73
Sugar	1.42	4.95
Proteids	0.91	3.90
Ash	0.21	0.80
Specific gravity	1.0090	1.0345

Infected milk may result from disease in the cow, or by after-infection by contact with diseased persons, the use of dirty vessels, etc. Tuberculosis, diphtheria, scarlet and typhoid fevers are all milk-borne diseases, and as milk forms a most favourable medium for rapid development, the organisms when once present increase with alarming rapidity, except in a few cases, such as the bacillus of tuberculosis, which does not increase in milk.

Milk may also be contaminated by non-pathogenic organisms which cause marked changes in the physical character of the milk. Chromogenic bacteria are sometimes present in the milk causing the conditions known as blue milk (due to bacillus cyanogenus), red milk (due to bacillus erythrogenus), and yellow milk (due to bacillus synxanthus). The fermentation in blue milk is marked by the production of a blue colour, which is changed to cherry red by alkalies, but the blue colour is restored by acids. The bacillus may be identified by cultures from the small patches formed in the milk; on gelatine plates it forms rounded, dirty-white, finely granular colonies with smooth outlines; the surrounding gelatine takes on a light-green or greenish-brown colour. In a primitive culture on gelatine, the remaining gelatine is greenish-blue, sometimes nearly black. On potatoes it forms a yellowish layer near the point of inoculation, the surrounding potato being stained blue. It is an ærobic and exceedingly mobile bacillus of 1 to 4 μ in length and 0.3 to 0.5 μ in breadth, with numerous flagellæ, and, when spores are present, club-shaped ends. Spores are frequently present in the middle as well as the ends of the rods. Grown in milk soured by lactic acid, it causes an intense blue coloration.

Ropy milk is due to the presence of organisms, which cause the milk to become very viscid and stringy, so that it may be drawn up in threads by a spoon.

Diseased milk is a subject more for the veterinary surgeon and the pathologist than for the analyst, but the detection of the bacillus of tuberculosis is frequently asked of the analyst, so that a few words on the subject may not be out of place.

To show the presence of tubercle bacilli in tuberculous milk, obtain the sediment for examination, by passing through a centrifugal apparatus. The sediment will be found to contain almost the whole of the bacilli with the mucus and solid particles. When the apparatus is unobtainable the best plan is to allow the milk to stand in a funnel-shaped separator, for about twenty-four hours. The sediment collected

at the bottom of the separator can be drawn off by means of the tap and a drop dried on a glass slide.

The preparations are stained in a solution made as follows:—

Take 1 part fuchsin and dissolve it in 10 parts of absolute alcohol, then add 100 parts of a 5 per cent solution of carbolic acid and heat the mixture until it steams freely. It takes three or four minutes, or even less, to stain cover glass preparations, whilst seven or eight minutes are necessary for the staining of sections. After getting rid of the superfluous fluid place the preparations in 90 per cent alcohol for a second or two, then plunge into a 25 per cent solution of sulphuric acid, when it will be noticed that the pinkish tinge has become a yellowish-brown. Wash the preparations in alcohol, and if they have sufficiently changed colour, place in water holding lithium carbonate in suspension. This process being completed they may be stained with a dilute solution of methylene blue.

THE ANALYSIS OF MILK.

The usual determinations in milk analysis involve the following:—

Specific gravity.

Total solid matter.

Fat.

Mineral matter.

Sugar.

Proteins.

Unless required for special purposes, the last three determinations are not usually made in the analysis of samples under the Food and Drugs Act, as the first three are usually sufficient to decide as to the purity of a sample.

Specific Gravity.—Pure normal milk rarely has a specific gravity below 1·031, sometimes rising to 1·035.

Total Solid Matter.—The legal minimum (see p. 41) for the total solid matter in milk is 11·5 per cent, but it is rarely that a genuine milk falls so low, 12·5 to 13·5 per cent covering the majority of pure samples, although occasionally 15 per cent will be found.

Determination of the Total Solids of Milk.—About 5 grms. of the milk are dried on a water bath in a small platinum capsule to constant weight and the residue weighed. The milk should preferably be weighed, although the error is very small if 5 c.c. be delivered from an accurate pipette and the result obtained divided by the specific gravity of the milk. The dishes should be flat-bottomed and the time of heating required will be, usually, about 5 hours for 5 grms. A skin forms over the surface of the milk as it dries making it somewhat difficult for the water to escape. This may be broken by a fine needle from time to time. Allen and Stokes prefer the use of porcelain dishes for the determination of the milk solids. Numerous devices to accelerate the drying of milk for the determination of the solid matter have been proposed, but they are of no practical advantage. For two of these reference may be made to the "Analyst" (xvii. 227, xvii. 79).

The use of a little recently ignited sand, or asbestos, accelerates the drying of the milk, however.

Determination of the Mineral Matter of Milk.—The platinum dish containing the above solid residue is heated to a low red heat, cooled in a desiccator when all the organic matter is consumed, and weighed. Prolonged or excessive heat causes a slightly low result to be obtained owing to the volatilization of chlorides. The ash of normal milk amounts to about 8 per cent of the non-fatty solids, or say, from 0.68 per cent to 0.78 per cent of the milk.

Determination of the Fat.—There are numerous methods of determining the fat in milk, but only a few of these will be described, many others being fully described in textbooks devoted to milk only.

(a) The Adams method. This method has the advantage of probably being the most accurate process known. A strip of fat-free absorbent paper about 2 inches wide and 24 inches long is rolled loosely into a coil and held by a wire, so that it can be conveniently suspended. Either 5 c.c. of milk are delivered by a pipette, slowly on to the coil, so that every drop is absorbed, or a beaker containing about 5 c.c. of milk is accurately weighed and the coil inserted, and when as much as possible is absorbed, the beaker is re-weighed, so that the amount of milk used is known. The coil is now hung up and air-dried and then transferred for a short time to the water oven. When completely dry it is transferred to the Soxhlet extraction apparatus (Fig. 3).

The coil is extracted for two to three hours, the tared flask containing the fat being heated at first on the water bath, and finally in the air oven, until of constant weight.

Soxhlet's ærometric process is as follows: 200 c.c. of milk are run into the flask H, and 10 c.c. of normal potash solution added, and 60 c.c. of ether, which has been saturated with water. The mixture is well shaken in the closed flask for fifteen minutes, and the liquids allowed to separate. By working the rubber bellows, sufficient of the ether solution may be transferred to the tube B, which is water-jacketed, to float the special form of hydrometer called the ærometer. The tube connecting the flask H with B is now closed by the clip. The

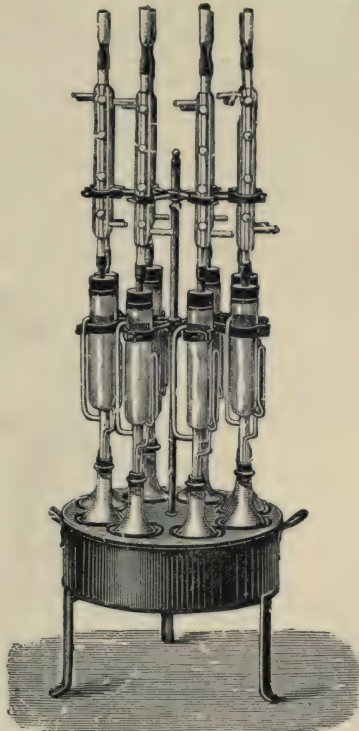


FIG. 3.—Soxhlet apparatus for milk fat.

amount of fat is determined by taking the reading of the ærometer, when the water jacket is exactly at 17.5°C . The reading then corresponds with the quantities of fat found in the annexed tables.

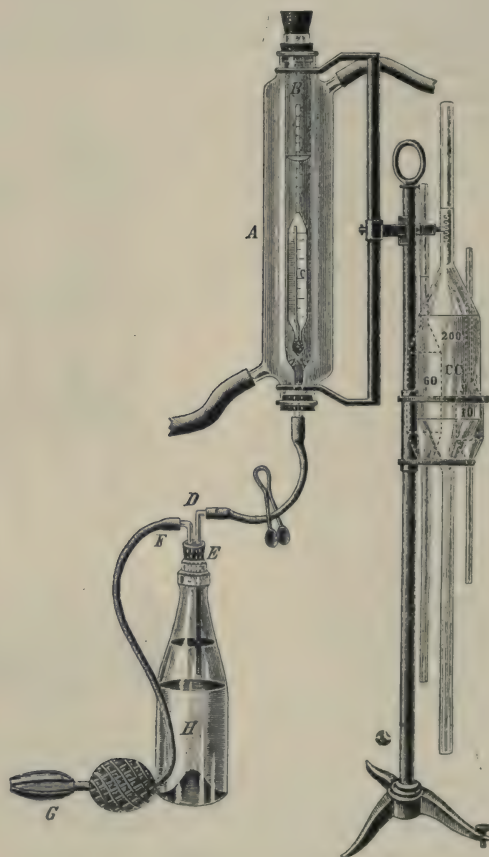


FIG. 4.—Soxhlet's ærometric apparatus.

This process is not used in this country, but is still employed to a small extent on the Continent.

SOXHLET'S FAT TABLE.

(In the specific gravities given here the figure 7 is omitted all through, as the small aerometer is thus graduated. Thus 21.1 implies a gravity of 0.7211.)

Sp. gr. at 17.5°.	Fat per cent	Sp. gr. at 17.5°.	Fat per cent	Sp. gr. at 17.5°.	Fat per cent	Sp. gr. at 17.5°.	Fat per cent	Sp. gr. at 17.5°.	Fat per cent
21.1	0.00	25.5	0.41	29.9	0.82	34.3	1.22	38.7	1.64
21.2	0.01	25.6	0.42	30	0.83	34.4	1.23	38.8	1.65
21.3	0.02	25.7	0.43	30.1	0.84	34.5	1.24	38.9	1.66
21.4	0.03	25.8	0.44	30.2	0.85	34.6	1.24	39	1.67
21.5	0.04	25.9	0.45	30.3	0.86	34.7	1.25	39.1	1.68
21.6	0.05	26	0.46	30.4	0.87	34.8	1.26	39.2	1.69
21.7	0.05	26.1	0.47	30.5	0.88	34.9	1.27	39.3	1.70
21.8	0.07	26.2	0.48	30.6	0.88	35	1.28	39.4	1.71
21.9	0.08	26.3	0.49	30.7	0.89	35.1	1.29	39.5	1.72
22	0.09	26.4	0.50	30.8	0.90	35.2	1.30	39.6	1.73
22.1	0.10	26.5	0.50	30.9	0.91	35.3	1.31	39.7	1.74
22.2	0.11	26.6	0.51	31	0.92	35.4	1.32	39.8	1.75
22.3	0.12	26.7	0.52	31.1	0.93	35.5	1.33	39.9	1.76
22.4	0.13	26.8	0.53	31.2	0.94	35.6	1.33	40	1.77
22.5	0.14	26.9	0.54	31.3	0.95	35.7	1.34	40.1	1.78
22.6	0.15	27	0.55	31.4	0.95	35.8	1.35	40.2	1.79
22.7	0.16	27.1	0.56	31.5	0.96	35.9	1.36	40.3	1.80
22.8	0.17	27.2	0.57	31.6	0.97	36	1.37	40.4	1.81
22.9	0.18	27.3	0.58	31.7	0.98	36.1	1.38	40.5	1.82
23	0.19	27.4	0.59	31.8	0.99	36.2	1.39	40.6	1.83
23.1	0.20	27.5	0.60	31.9	1.00	36.3	1.40	40.7	1.84
23.2	0.21	27.6	0.60	32	1.01	36.4	1.41	40.8	1.85
23.3	0.22	27.7	0.61	32.1	1.02	36.5	1.42	40.9	1.86
23.4	0.23	27.8	0.62	32.2	1.03	36.6	1.43	41	1.87
23.5	0.24	27.9	0.63	32.3	1.04	36.7	1.44	41.1	1.88
23.6	0.25	28	0.64	32.4	1.05	36.8	1.45	41.2	1.89
23.7	0.25	28.1	0.65	32.5	1.05	36.9	1.46	41.3	1.90
23.8	0.26	28.2	0.66	32.6	1.06	37	1.47	41.4	1.91
23.9	0.27	28.3	0.67	32.7	1.07	37.1	1.48	41.5	1.92
24	0.28	28.4	0.68	32.8	1.08	37.2	1.49	41.6	1.93
24.1	0.29	28.5	0.69	32.9	1.09	37.3	1.50	41.7	1.94
24.2	0.30	28.6	0.70	33	1.10	37.4	1.51	41.8	1.95
24.3	0.30	28.7	0.71	33.1	1.11	37.5	1.52	41.9	1.96
24.4	0.31	28.8	0.72	33.2	1.12	37.6	1.53	42	1.97
24.5	0.32	28.9	0.73	33.3	1.13	37.7	1.54	42.1	1.98
24.6	0.33	29	0.74	33.4	1.14	37.8	1.55	42.2	1.99
24.7	0.34	29.1	0.75	33.5	1.15	37.9	1.56	42.3	2.00
24.8	0.35	29.2	0.76	33.6	1.15	38	1.57	42.4	2.01
24.9	0.36	29.3	0.77	33.7	1.16	38.1	1.58	42.5	2.02
25	0.37	29.4	0.78	33.8	1.17	38.2	1.59	42.6	2.03
25.1	0.38	29.5	0.79	33.9	1.18	38.3	1.60	42.7	2.04
25.2	0.39	29.6	0.80	34	1.19	38.4	1.61	42.8	2.05
25.3	0.40	29.7	0.80	34.1	1.20	38.5	1.62	42.9	2.06
25.4	0.40	29.8	0.81	34.2	1.21	38.6	1.63	43	2.07

Sp. gr. at 17°50.	Fat per cent	Sp. gr. at 17°50.	Fat per cent	Sp. gr. at 17°50.	Fat per cent	Sp. gr. at 17°50.	Fat per cent	Sp. gr. at 17°50.	Fat per cent
43	2.07	47.7	2.61	52.3	3.16	56.9	3.74	61.5	4.39
43.1	2.08	47.8	2.62	52.4	3.17	57	3.75	61.6	4.40
43.2	2.09	47.9	2.63	52.5	3.18	57.1	3.76	61.7	4.42
43.3	2.10	48	2.64	52.6	3.20	57.2	3.78	61.8	4.44
43.4	2.11	48.1	2.66	52.7	3.21	57.3	3.80	61.9	4.46
43.5	2.12	48.2	2.67	52.8	3.22	57.4	3.81	62	4.47
43.6	2.13	48.3	2.68	52.9	3.23	57.5	3.82	62.1	4.48
43.7	2.14	48.4	2.70	53	3.25	57.6	3.84	62.2	4.50
43.8	2.16	48.5	2.71	53.1	3.26	57.7	3.85	62.3	4.52
43.9	2.17	48.6	2.72	53.2	3.27	57.8	3.87	62.4	4.53
44	2.18	48.7	2.73	53.3	3.28	57.9	3.88	62.5	4.55
44.1	2.19	48.8	2.74	53.4	3.29	58	3.90	62.6	4.56
44.2	2.20	48.9	2.75	53.5	3.30	58.1	3.91	62.7	4.58
44.3	2.22	49	2.76	53.6	3.31	58.2	3.92	62.8	4.59
44.4	2.23	49.1	2.77	53.7	3.33	58.3	3.93	62.9	4.61
44.5	2.24	49.2	2.78	53.8	3.34	58.4	3.95	63	4.63
44.6	2.25	49.3	2.79	53.9	3.35	58.5	3.96	63.1	4.64
44.7	2.26	49.4	2.80	54	3.37	58.6	3.98	63.2	4.66
44.8	2.27	49.5	2.81	54.1	3.38	58.7	3.99	63.3	4.67
44.9	2.28	49.6	2.83	54.2	3.39	58.8	4.01	63.4	4.69
45	2.30	49.7	2.84	54.3	3.40	58.9	4.02	63.5	4.70
45.1	2.31	49.8	2.86	54.4	3.41	59	4.03	63.6	4.71
45.2	2.32	49.9	2.87	54.5	3.43	59.1	4.04	63.7	4.73
45.3	2.33	50	2.88	54.6	3.45	59.2	4.06	63.8	4.75
45.4	2.34	50.1	2.90	54.7	3.46	59.3	4.07	63.9	4.77
45.5	2.35	50.2	2.91	54.8	3.47	59.4	4.09	64	4.79
45.6	2.36	50.3	2.92	54.9	3.48	59.5	4.11	64.1	4.80
45.7	2.37	50.4	2.93	55	3.49	59.6	4.12	64.2	4.82
45.8	2.38	50.5	2.94	55.1	3.51	59.7	4.14	64.3	4.84
45.9	2.39	50.6	2.96	55.2	3.52	59.8	4.15	64.4	4.85
46	2.40	50.7	2.97	55.3	3.53	59.9	4.16	64.5	4.87
46.1	2.42	50.8	2.98	55.4	3.55	60	4.18	64.6	4.88
46.2	2.43	50.9	2.99	55.5	3.56	60.1	4.19	64.7	4.90
46.3	2.44	51	3.00	55.6	3.57	60.2	4.20	64.8	4.92
46.4	2.45	51.1	3.01	55.7	3.59	60.3	4.21	64.9	4.93
46.5	2.46	51.2	3.03	55.8	3.60	60.4	4.23	65	4.95
46.6	2.47	51.3	3.04	55.9	3.61	60.5	4.24	65.1	4.97
46.7	2.49	51.4	3.05	56	3.63	60.6	4.26	65.2	4.98
46.8	2.50	51.5	3.06	56.1	3.64	60.7	4.27	65.3	5.00
46.9	2.51	51.6	3.08	56.2	3.65	60.8	4.29	65.4	5.02
47	2.52	51.7	3.09	56.3	3.67	60.9	4.30	65.5	5.04
47.1	2.54	51.8	3.10	56.4	3.68	61	4.32	65.6	5.05
47.2	2.55	51.9	3.11	56.5	3.69	61.1	4.33	65.7	5.07
47.3	2.56	52	3.12	56.6	3.71	61.2	4.35	65.8	5.09
47.4	2.57	52.1	3.14	56.7	3.72	61.3	4.36	65.9	5.11
47.5	2.58	52.2	3.15	56.8	3.73	61.4	4.37	66	5.12
47.6	2.60								

The Werner-Schmidt Process.—Ten grms. of milk are weighed into a 50 c.c. stout glass tube, about 8 inches long, and 10 c.c. of strong hydrochloric acid added. The tube is then placed in boiling water until the contents become dark brown, which usually takes place in about ten minutes. The tube is then cooled by immersing it in cold water,

and 30 c.c. of ether added. The whole is well shaken and the ether transferred to a tared flask by means of a pipette (or more easily by closing the tube with a cork pierced by tubes similar to those used in a wash-bottle, of suitable length); the shaking with ether is repeated three times, the ether evaporated and the fat weighed. This process is particularly applicable when the milk has become sour.

Gottlieb has modified a method devised by R  se for the determination of fat by extraction with ether and petroleum ether from an alkaline solution of milk ("Chem. Zeit." xxii. 632).

Ten c.c. of milk, 1 c.c. of 20 per cent ammonia, and 10 c.c. of 95 per cent alcohol are shaken in a glass tube about 40 cm. long. Twenty-five c.c. of ether are added, and the tube is inverted several times. Then 25 c.c. of petroleum ether are added, and the whole is well agitated. After complete separation the ethereal layer is removed, and the milk again extracted with ether and petroleum ether, and the second extract mixed with the first. This process is again twice repeated. The solvent is evaporated and the fat dried at 100  and weighed. The fat should be dissolved in a little petroleum ether, and the small residue of non-fatty solids subtracted from the weight.

The essential point of this method is the complete mixing of each solvent before the addition of the next.

Bell's Process.—This depends on the evaporation of 10 grms. of the milk with constant stirring, until a not too dry, pulverulent, residue is obtained. This is repeatedly treated with warm ether which is filtered into a tared beaker, the last traces of fat being washed through the paper with more ether, the ether evaporated and the residue weighed.

Centrifugal Separation of the Fat.—In all processes of this type, the milk is whirled in a centrifugal apparatus in closed tubes, after being treated by a suitable reagent. The two most useful instruments are those of Gerber and Leffmann-Beam, which are modifications of a process devised by Babcock.

Leffmann-Beam Process.—H. Leffmann and W. Beam were the originators of this method of fat-separation. The centrifugal apparatus used was made by Beimling. This apparatus is made to hold four, eight or twelve bottles according to the number required. In using it take 15 c.c. of the sample of milk and pour it into the specially constructed 30 c.c. bottle which has a long graduated neck. Each division

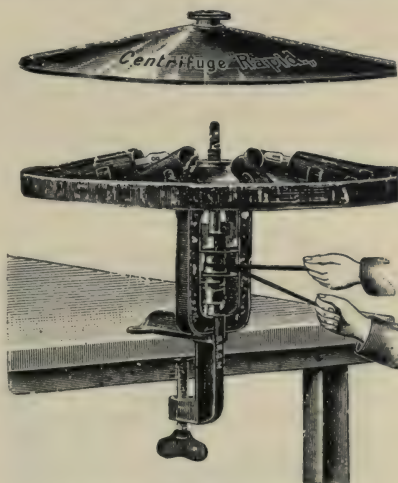


FIG. 5.—Centrifugal apparatus.

represents 0.10 per cent of milk-fat. Then add 3 c.c. of a mixture of equal parts of amylac alcohol and fuming hydrochloric acid (sp. gr. 1.16). Shake well and slowly add sulphuric acid (sp. gr. 1.835) shaking all the time. The contents are now hot, and the casein which was previously separated, now completely dissolves, and the liquid becomes a dark reddish-brown colour. Mix two measures of water with one of strong sulphuric acid and add until the liquid reaches the zero mark on the bottle neck. Rotate the bottle for two minutes in the centrifugal machine. If the sample of milk used is poor in fat, or is skimmed milk, the rotation should be continued a minute or two longer. It will be noticed on stopping the machine that there is a layer of fat in the neck of the bottle, the amount of which can be ascertained from the graduations. To do this accurately take a pair of dividers, the legs of which should be placed at the upper and lower limits of the layer of fat, allowance being made for the meniscus. Then move the dividers until one point is on the zero mark of the scale. The percentage of fat will be shown from the position of the other leg.

The amount of fat in cream can also be estimated by the Leffmann-Beam process. Take about 2 c.c. of the sample, put in the bottle and add 15 c.c. of water. Multiply the reading by 15.25 and divide by the weight (in grammes) of the sample taken. If only one test is being made, the arms of the machine should be balanced by either a duplicate test, or a bottle filled with diluted sulphuric acid, placed in the carrier opposite the one containing the sample.

Gerber Process.—Another form of the centrifugal apparatus is that devised by N. Gerber. He directs in the instructions issued with the instrument, that 10 c.c. of sulphuric acid, specific gravity not less than 1.820 or more than 1.825, should be poured into one of the bottles. Eleven c.c. of the milk to be tested is then added, and finally 1 c.c. of amylac alcohol, care being taken to pour gently down the side of the bottle. Insert a tightly fitting india-rubber stopper in the bottle neck

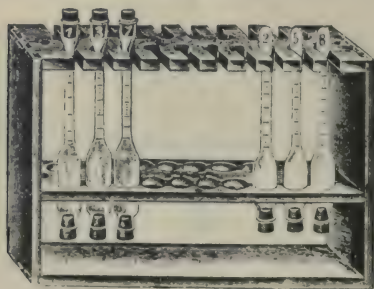


FIG. 6.—Bottles for fat separation in milk.



FIG. 7 —Babcock bottles.

and shake well until the contents are thoroughly mixed. Place the bottle, while still hot, in the rotator, screw the top on, and, by pulling the cat-gut string make the whole revolve as quickly as possible. It will take about two or three minutes to separate the fat, after which

the bottle should be plunged into water at 60° to 70° C. and the volume of fat ascertained from the graduations. When the sample is skimmed milk the rotation and immersion in warm water should be repeated several times before reading off the amount of fat. The same treatment would be used for condensed milk, which should be first diluted with nine times its weight of water.

The following table gives the necessary amounts of milk and reagents required in the centrifugal methods of fat separation :—

	Babcock.	Leffmann - Beam.	Gerber.	Stokes.
Milk	17.5 c.c.	15 c.c.	11 c.c.	15 c.c.
Sulphuric acid (volume)	17.5 "	9 "	10 "	13.5 "
Sulphuric acid (specific gravity) .	1.831 to 1.834	1.835	1.820 to 1.825	1.820 to 1.830
Hydrochloric acid	none	1.5 c.c.	none	none
Amylic alcohol	"	1.5 "	1.0 c.c.	1.5 c.c.

Wollny ("Milch. Zeit." 1900, 50) recommends the determination of the refractive index of an ethereal solution of the fat obtained under definite conditions, given refractive indices (or arbitrary readings on the refractometer) corresponding to given fat values. The grave objection to this process is that during the transference of the few drops of ether solution to the refractometer, loss of ether may easily occur by evaporation, and thus too high results be obtained.

There is a fairly constant relation between the specific gravity, total solids, and fat in a sample of milk and Droop Richmond and Hehner ("Analyst," XIII. 32) have adopted the following formula from which the amount of fat can be approximately calculated when the specific gravity and solids are known :—

$$T = 1.164 F + 0.254 G,$$

when T is the total solids, F the fat, and G the specific gravity. Several modified formulæ have been proposed but none remove the calculation outside the limit of experimental error, so that they are but slight improvements, if any, on the above. According to Allen, Richmond now prefers :—

$$P = 1.2 F + 0.14 + 0.25 G.$$

The table on page 54 is based on Richmond and Hehner's formula. The figures in the body of the table are the total solids.

Determination of the Milk Sugar in Milk.—The only practical, and, at the same time, accurate, methods of determining the amount of sugar in milk are based on the optical rotatory power of that substance, or on its power of reducing cupric oxide. Heat has a variable effect, according to circumstances, on the optical rotation of milk sugar or lactose, but does not affect its reducing power. Hence sugar cannot be very accurately estimated by the polarimeter if the milk has been boiled.

(A) *Polarimetric Determination.*—It is first necessary to obtain a

TABLE BASED ON HEHNER & RICHMOND'S MILK FORMULA.

Specific Gravity.	Percentage of Fat.																			
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
1024.0	8.42	8.54	8.66	8.77	8.89	9.01	9.12	9.24	9.35	9.47	9.59	9.70	9.82	9.94	10.05	10.17	10.29	10.40	10.52	10.63
1025.0	8.56	8.68	8.79	8.90	9.02	9.14	9.25	9.37	9.48	9.60	9.72	9.83	9.95	10.07	10.18	10.30	10.42	10.53	10.65	10.76
1026.0	8.68	8.80	8.91	9.02	9.14	9.26	9.37	9.49	9.60	9.72	9.84	9.95	10.07	10.18	10.30	10.42	10.54	10.65	10.77	10.88
1027.0	8.81	8.93	9.04	9.15	9.27	9.39	9.50	9.62	9.73	9.85	9.97	10.08	10.20	10.32	10.44	10.55	10.67	10.78	10.90	11.01
1028.0	8.94	9.06	9.17	9.28	9.40	9.52	9.63	9.75	9.86	9.98	10.10	10.21	10.33	10.45	10.56	10.68	10.80	10.91	11.03	11.14
1029.0	9.07	9.19	9.31	9.42	9.53	9.65	9.77	9.88	10.00	10.11	10.23	10.35	10.46	10.58	10.70	10.81	10.93	11.04	11.16	11.28
1030.0	9.20	9.32	9.44	9.55	9.66	9.78	9.90	10.01	10.13	10.24	10.36	10.48	10.59	10.71	10.83	10.94	11.06	11.18	11.29	11.41
1031.0	9.33	9.45	9.57	9.68	9.79	9.91	10.03	10.14	10.26	10.37	10.49	10.61	10.72	10.84	10.96	11.07	11.19	11.31	11.42	11.54
1032.0	9.46	9.58	9.70	9.81	9.92	10.04	10.16	10.27	10.38	10.49	10.61	10.72	10.84	10.96	11.08	11.19	11.31	11.43	11.54	11.66
1033.0	9.59	9.71	9.83	9.94	10.05	10.17	10.28	10.39	10.50	10.61	10.72	10.84	10.96	11.08	11.19	11.31	11.43	11.54	11.66	11.77
1034.0	9.72	9.84	9.96	10.07	10.18	10.29	10.40	10.51	10.62	10.73	10.84	10.96	11.08	11.19	11.31	11.43	11.54	11.66	11.77	11.89
1035.0	9.85	9.97	10.09	10.20	10.31	10.42	10.53	10.64	10.75	10.86	10.97	11.08	11.19	11.31	11.43	11.54	11.66	11.77	11.89	12.01
1036.0	9.98	10.10	10.22	10.33	10.44	10.55	10.66	10.77	10.88	10.99	11.10	11.21	11.32	11.43	11.54	11.66	11.77	11.89	12.01	12.13
1037.0	10.11	10.23	10.35	10.46	10.57	10.68	10.79	10.90	11.01	11.12	11.23	11.34	11.45	11.56	11.67	11.78	11.89	12.01	12.13	12.25
1038.0	10.24	10.36	10.48	10.59	10.70	10.81	10.92	11.03	11.14	11.25	11.36	11.47	11.58	11.69	11.80	11.91	12.02	12.13	12.25	12.37
1039.0	10.37	10.49	10.61	10.72	10.83	10.94	11.05	11.16	11.27	11.38	11.49	11.60	11.71	11.82	11.93	12.04	12.15	12.26	12.37	12.49
1040.0	10.50	10.62	10.74	10.85	10.96	11.07	11.18	11.29	11.40	11.51	11.62	11.73	11.84	11.95	12.06	12.17	12.28	12.39	12.50	12.61
1041.0	10.63	10.75	10.87	10.98	11.09	11.20	11.31	11.42	11.53	11.64	11.75	11.86	11.97	12.08	12.19	12.30	12.41	12.52	12.63	12.74
1042.0	10.76	10.88	11.00	11.11	11.22	11.33	11.44	11.55	11.66	11.77	11.88	11.99	12.10	12.21	12.32	12.43	12.54	12.65	12.76	12.87
1043.0	10.89	11.01	11.13	11.24	11.35	11.46	11.57	11.68	11.79	11.90	12.01	12.12	12.23	12.34	12.45	12.56	12.67	12.78	12.89	13.01
1044.0	11.02	11.14	11.26	11.37	11.48	11.59	11.70	11.81	11.92	12.03	12.14	12.25	12.36	12.47	12.58	12.69	12.80	12.91	13.02	13.14
1045.0	11.15	11.27	11.39	11.50	11.61	11.72	11.83	11.94	12.05	12.16	12.27	12.38	12.49	12.60	12.71	12.82	12.93	13.04	13.15	13.27
1046.0	11.28	11.40	11.52	11.63	11.74	11.85	11.96	12.07	12.18	12.29	12.40	12.51	12.62	12.73	12.84	12.95	13.06	13.17	13.28	13.40
1047.0	11.41	11.53	11.65	11.76	11.87	11.98	12.09	12.20	12.31	12.42	12.53	12.64	12.75	12.86	12.97	13.08	13.19	13.30	13.41	13.53
1048.0	11.54	11.66	11.78	11.89	12.00	12.11	12.22	12.33	12.44	12.55	12.66	12.77	12.88	12.99	13.10	13.21	13.32	13.43	13.54	13.66
1049.0	11.67	11.79	11.91	12.02	12.13	12.24	12.35	12.46	12.57	12.68	12.79	12.90	13.01	13.12	13.23	13.34	13.45	13.56	13.67	13.79
1050.0	11.80	11.92	12.04	12.15	12.26	12.37	12.48	12.59	12.70	12.81	12.92	13.03	13.14	13.25	13.36	13.47	13.58	13.69	13.80	13.92
1051.0	11.93	12.05	12.17	12.28	12.39	12.50	12.61	12.72	12.83	12.94	13.05	13.16	13.27	13.38	13.49	13.60	13.71	13.82	13.93	14.05
1052.0	12.06	12.18	12.30	12.41	12.52	12.63	12.74	12.85	12.96	13.07	13.18	13.29	13.40	13.51	13.62	13.73	13.84	13.95	14.06	14.18
1053.0	12.19	12.31	12.43	12.54	12.65	12.76	12.87	12.98	13.09	13.20	13.31	13.42	13.53	13.64	13.75	13.86	13.97	14.08	14.19	14.31
1054.0	12.32	12.44	12.56	12.67	12.78	12.89	13.00	13.11	13.22	13.33	13.44	13.55	13.66	13.77	13.88	13.99	14.10	14.21	14.32	14.44
1055.0	12.45	12.57	12.69	12.80	12.91	13.02	13.13	13.24	13.35	13.46	13.57	13.68	13.79	13.90	14.01	14.12	14.23	14.34	14.45	14.57

clear solution of the sugar. Wiley (Bulletin 13, United States Dept. of Agriculture) has examined this subject exhaustively, and recommended the use of an acid solution of mercuric nitrate to clarify the milk. This has the advantage over acetic acid and lead acetate as recommended by Schmoeger, since heat is required in the latter process. Various other precipitants may be used, but as mercuric nitrate is the most satisfactory, the others need not be discussed. Richmond, however, has shown ("Analyst," xxxv. 576) that traces of proteids are left in solution, and he adds 5 per cent of phosphotungstic acid and 5 per cent of 50 per cent H_2SO_4 . This ensures complete precipitation, and by allowing for the increase in volume, the polarimetric reading gives the amount of lactose at once.

Lactose is estimated by determining the optical activity of its solution. When applying this method to milk, first prepare a clear whey free from other optically active substances. Wiley ("Amer. Chem. J." 1884, 6, No. 5.) proves that precipitation by basic lead acetate does not completely remove the l  vorotatory protein matters; he suggests two alternative mercurial reagents. His method, which has been officially adopted in America, is as follows:—

(a) *Acid Mercuric Nitrate*.—Dissolve mercury in twice its weight of nitric acid, specific gravity 1.42, and dilute with an equal volume of water. 1 c.c. of this reagent is sufficient for the quantities of milk mentioned below. Larger quantities may be used without affecting the results of polarization.

(b) *Mercuric Iodide with Acetic Acid*.—Mix 33.2 grms. of potassium iodide with 13.5 grms. of mercuric chloride, 20 c.c. of glacial acetic acid and 640 c.c. of water.

Estimation.—The milk should be kept at one temperature and its specific gravity determined.

The quantities of milk measured for polarization vary with the specific gravity of the milk and also with the polariscope used. The quantity to be measured will be found in any case in the accompanying table:—

Specific gravity.	Volume of Milk to be used.	
	For Polariscopes of which the Sucrose Normal Weight is 16.19 grammes.	For Polariscopes of which the Sucrose Normal Weight is 26.048 grammes.
	c.c.	c.c.
1.024	60.0	64.4
1.026	59.9	64.3
1.028	59.8	64.15
1.030	59.7	64.0
1.032	59.6	63.9
1.034	59.5	63.8
1.035	59.35	63.7

Take a flask graduated at 102.4 c.c. for a Laurent or 102.6 c.c. for a Ventzke polariscope and place in it the quantity of milk given in the table. Add 1 c.c. of mercuric nitrate solution or 30 c.c. of mercuric iodide solution (an excess of those reagents does no harm), fill to the mark, shake well, filter through a dry filter and polarize. There is no necessity to heat before polarizing. If a 200 mm. tube is used, divide the polariscope reading by 3 when the sucrose normal weight for the instrument is 16.19 grms. or by 2 when the normal weight for the instrument is 26.048. The lactose normal weight to read 100° on the sugar scale for Laurent instruments is 20.496 grms. and for Ventzke instruments 32.975 grms.

Wiley and Ewell in a later paper prefer to use the method of double dilution, which does away with the necessity for allowing for the volume of precipitated matter. They add 4 c.c. of the acid mercuric nitrate solution to two portions of the milk and dilute one of these to 100 c.c. and the other to 200 c.c. The liquids are filtered and the optical rotation taken in a 400 mm. tube. The reading of the dilute solution is multiplied by two, and the product subtracted from the reading of the stronger solution, the difference being called *a*. Then, the reading of the stronger solution - 2 *a*, divided by four (assuming a Soleil-Ventzke instrument is used) gives the percentage of lactose. A little consideration will show that this is a mathematically correct proportion.

Rupp recommends treating 50 c.c. of milk with 25 c.c. of lead acetate solution (10 per cent), heating, filtering, and when cool making up to 100 c.c. Each angular degree of rotation in 100 mm. tube corresponds to 0.205 gm. of milk sugar. In the author's experience 0.195 is the more correct value per 1°. A simpler process is described by Vieth ("Analyst," XIII. 63) but in the author's opinion is not so accurate.

(B) *Volumetric determination.*

Milk sugar may be determined by reduction of Fehling's solution. In order to prepare the milk for the action of Fehling's solution, 25 grms. are mixed with 0.5 c.c. of 30 per cent acetic acid solution and the mixture well shaken and 100 c.c. of boiling water is added; 25 c.c. of recently made cream of alumina are then added (cream of alumina is prepared by adding a slight excess of NH_3 to a saturated alum solution, and then adding more alum till faintly acid), the whole well shaken and allowed to stand for fifteen minutes. The liquid is then filtered through a moistened filter paper and the filtrate and washings made up to 250 c.c. The liquid must be quite clear. The liquid is now titrated in the usual manner (see p. 123) against 10 c.c. of Fehling's solution, or the precipitated copper is weighed as cupric oxide. If the gravimetric process be used, 25 c.c. of the milk solution should be boiled with 30 c.c. of Fehling's solution and 50 c.c. of water. For the purposes of calculation, it may be taken that 0.067 gm. of lactose reduces 10 c.c. of Fehling's solution, or that the weight of cupric oxide $\times 0.6024$ will represent anhydrous lactose. If more accurate results be required the following table gives the amount of lactose corresponding to given weights of metallic copper (which can be calculated from the cupric oxide):—

Copper.	Lactose.	Copper.	Lactose.	Copper.	Lactose.
120 =	86.4	220 =	161.9	310 =	232.2
130 =	93.8	230 =	169.4	320 =	240.0
140 =	101.3	240 =	176.9	330 =	247.7
150 =	108.8	250 =	184.8	340 =	255.1
160 =	116.4	260 =	192.5	350 =	263.9
170 =	123.9	270 =	200.3	360 =	272.1
180 =	131.6	280 =	208.3	370 =	280.5
190 =	139.3	290 =	216.3	380 =	289.1
200 =	146.9	300 =	228.3	390 =	297.7
210 =	154.5			400 =	306.3

Intermediate results can be interpolated.

Determination of the Proteids of Milk.—A determination of the total nitrogen by Kjeldahl's process ($N \times 6.33$) gives a sufficiently accurate result for the proteids in milk, as practically the whole of the nitrogen in milk exists in the form of proteids. The determination should be made in the following manner:—

Three c.c. of the milk are heated in a hard Jena glass round-bottomed flask with 20 c.c. of strong sulphuric acid, with the addition of a small globule of mercury, as recommended by Dyer. The liquid is allowed to boil briskly and in ten minutes 10 grms. of potassium sulphate are added, and the boiling is continued until the contents of the flask are clear and nearly colourless. The contents of the flask are allowed to cool and transferred to a large distilling flask (round-bottomed Jena glass flasks are preferable). The flask having a cork with two holes in it, is connected with any convenient condenser and has a tap-funnel attached through the second hole. Though this caustic soda solution is cautiously added in slight excess (as indicated by means of the addition of a drop of phenol-phthalein solution to the soda solution, which is run in until a pink colour is permanent, when a further few c.c. should be added). A little pumice stone is added to prevent bumping. If mercury has been used, a little sodium sulphide should be added to the distilling flask in order to decompose any compound of mercury and nitrogen, but the mercury is not essential and may be omitted. The distillation is carefully conducted, the distillate being received in the usual manner into an excess of decinormal sulphuric acid (30 c.c.). Each c.c. of acid neutralized, as determined by titration, corresponds to 0.0014 grm. of nitrogen or 0.00886 grm. of milk proteids. A blank experiment is necessary to allow for the traces of nitrogen present in the reagents. A tin condensing tube is better than glass, as there may be some action of the steam on the glass.

(For further details of the Kjeldahl process see "Trans. Chem. Soc." 1895, 118).

Richmond and Bosely ("Analyst" xviii. 172) recommend the following method: 10 grms. of milk are diluted with about 200 c.c. of water, and rendered faintly alkaline (to phenol-phthalein) with dilute caustic soda solution. From 2 to 2.5 c.c. of a 6 per cent solution of copper sulphate are then added, the mixture shaken, and then allowed to settle. The precipitate is washed five times by decantation, the washings being poured through a tared filter paper. It is then transferred to the filter, washed once or twice more with water, dried for a short time in a water oven, extracted with ether to remove traces of fat

and dried at 130°, and weighed. It is afterwards burnt and the ash deducted from the original weight. The result is the sum of the casein and albumen. In practice, the author prefers Muter's suggestion of not drying the precipitate before washing with ether, but of washing it with absolute alcohol and then directly afterwards with ether. Rupp recommends using 10 grms. of milk and 100 to 150 c.c. of water, to which is added 15 c.c. of a 6 per cent solution of copper sulphate; 7 c.c. of a 1·5 c.c. of caustic soda are then added and the precipitate of proteids, copper hydroxide and a little fat is transferred to a tared filter paper, and washed successively with water (several times) alcohol and ether. It is then dried and weighed and the ash deducted from the weight. The disadvantage of this method is that a relatively large amount of copper is precipitated, and the ash is very high as compared with the amount of precipitate.

Sebelien separates the casein and albumin in the following manner: About 10 grms. of milk are mixed with double its volume of a saturated solution of magnesium sulphate, and then crystals of the salt added so long as they dissolve on shaking. The liquid is allowed to stand for three to four hours and the precipitate is washed with a saturated solution of magnesium sulphate. The precipitate with the filter paper is treated with 30 c.c. of strong sulphuric acid and the nitrogen determined by Kjeldahl's process. The nitrogen $\times 6\cdot33$ may be taken as representing the casein. The albumin, which is in the filtrate is precipitated by a solution of phosphotungstic acid, and the nitrogen in the precipitate determined. A simple and comparatively accurate process for the estimation of the casein and albumin is to dilute 10 to 20 c.c. of milk with ten times its volume of water and acidify with a drop or two of acetic acid. The mixture is now warmed to 40° C. and the resulting precipitate of casein transferred to a tared filter, washed with water, alcohol, and ether, dried and weighed. The filtrate is further acidified with a little acetic acid and heated to boiling-point. The precipitate of albumin formed is collected on a tared filter, washed with water, dried and weighed. Greater accuracy is obtained by determining the nitrogen in the precipitates, using the factor $N \times 6\cdot37 =$ casein, and $N \times 6\cdot73 =$ albumin.

CALCULATION OF ADULTERATION.

The calculation of the amount of adulteration of milk is necessarily based on an arbitrary standard for the original milk.

For legal purposes the solids other than fat must be present to the extent of at least 8·5 per cent (assuming the fat to be only 3 per cent). On the assumption of this figure as a standard, the formula

$$W = 100 - \frac{S \times 100}{8\cdot5}$$

will give the percentage of added water (i.e. calculated on the sample) when W is that percentage and S is the amount of non-fatty solids in the sample.

In cases where the adulteration is an abstraction of fat, the ap-

proximate percentage abstracted, on the same assumption as above, is calculated from the formula

$$P = \frac{100 (F_1 - F_2)}{F_1}$$

when P is the percentage (calculated on the total fat normally present, i.e. the legal minimum of 3 per cent) of fat abstracted, F_1 is the normal amount (i.e. 3 per cent) and F_2 is the amount in the sample.

POISONOUS MILK.

V. C. Vaughan named the poisonous ptomaine which he discovered in stale milk, ice-creams and cheese, tyrotoxinon. It crystallizes in needles which gradually decompose when exposed to moist air. Its odour is similar to that of stale cheese, and it has a "dry" taste. It is soluble in water, alcohol, and chloroform, but insoluble in ether when pure, though it dissolves if other impurities are present.

Vaughan found that tyrotoxinon was exceedingly poisonous both to man and the lower animals. The smallest portion placed on a child's tongue caused symptoms identical with those of cholera infantum, viz. sickness and diarrhoea. When ten drops of a solution of tyrotoxinon taken from milk three months old, were given to a young dog, it caused frothing at the mouth, vomiting, diarrhoea and muscular spasms. Cats exhibited the same symptoms. The mucous membrane of the stomach showed no inflammation after death, being white and soft.

When a strong solution of tyrotoxinon is evaporated with some platonic chloride on a water bath, a violent explosion takes place as soon as the whole of the alcohol has evaporated.

Tyrotoxinon does not precipitate with most of the general reagents for alkaloids.

Tyrotoxinon forms a potassium derivative, which crystallizes in six-sided plates, soluble in alcohol from which it is precipitated by ether.

Vaughan considers this compound is diazobenzene potassoxide $C_6H_5N_2OK$, and believes that tyrotoxinon if not identical with diazobenzene butyrate is closely related to it.

He suggests the following test for detecting tyrotoxinon in milk and cheese:—

Neutralize the filtrate from the curdled milk or the filtered cold water extract of cheese, with sodium carbonate, place in a separator and agitate with its own volume of ether. Allow the mixture to stand for twenty-four hours, or until separation has taken place, then leave the ethereal layer to evaporate spontaneously in an open dish. Dissolve the residue in water, again agitate the liquid with ether, separate the ethereal layer, and allow to evaporate as before. There should not be repeated extractions with ether, as the tyrotoxinon becomes less easy to dissolve the purer it is. Dissolve the residue in a few drops of distilled water and examine the solution as follows:—

(a) Place a drop of the liquid on porcelain with a few drops of a freshly prepared mixture of phenol and concentrated sulphuric acid, free from nitrous compounds. If tyrotoxinon is present there will be a

coloration varying from yellow to orange-red and finally becoming violet.

(b) Add a concentrated solution of caustic potash to the remainder of the solution and evaporate to dryness on the water bath. If tyrotoxin is present, diazobenzene-potassoxide will be formed, and can be recognized by its crystalline form and green colour produced on the addition of a mixture of phenol and strong sulphuric acid.

An acid solution of tyrotoxin prepared from milk gives with auric chloride a golden-yellow precipitate, but the gold salt forms very slowly from some milks, probably on account of the presence of other organic matter.

PRESERVATIVES IN MILK.

It is very frequently necessary to examine samples of milk for the presence of preservatives. Of these the principal are boric acid and formic aldehyde. Other preservatives are used from time to time, but the efficiency of the two above-mentioned have caused them to replace other preservatives almost entirely. M. Blyth ("Analyst," xxvi. 149) claims that the presence of preservatives in milk is accurately indicated by the following method.

Measure 10 c.c. of each milk into clean wide test tubes. Measure 10 c.c. of a sterile milk known to be free from preservatives into a test tube (these control tubes can be kept ready for use). Add to each milk and to the control 2 c.c. of a very strong, slightly alkaline solution of litmus. Now examine all the tubes, and if any of them are not the same shade of blue as the control tube, drop in, drop by drop, a half normal solution of sodium hydrate until the correct shade of blue is obtained. This will be found unnecessary in the case of most milks, and will only be requisite when the milks are two or three days old; this process must then be done very carefully. Plug all the tubes with cotton wool, and heat them in a water bath, kept at a temperature of 80° C. for ten minutes. Allow the tubes to cool, and inoculate each, including the control, with half a c.c. of sour milk in water (half c.c. milk to 100 c.c. water). Shake the tubes well. Now let the tubes stand for twenty-four hours at any temperature between 15° C. and 22° C., and then examine. If the control tube be not white, or nearly so, they must be allowed to stand for a longer period. Those tubes which contain preservatives will remain blue or pink, while the tubes which contain no preservatives will behave in the same way as the control tubes, becoming quite white. The length of time the blue or pink colour takes to become white depends upon the quantity of preservative present in the sample. The quantities of the more common preservatives which can be detected with certainty by this method, are 0.005 per cent of borax, boracic acid, or mixtures of these substances, 0.05 per cent of salicylic acid, and 0.0003 of formic aldehyde, quantities very much smaller than are ever found, or which would be of any value in commercial milks. Having selected by this method those samples which contain preservatives, the nature of these must be determined by the ordinary methods.

Boric acid, borates, and a mixture of boric acid and borax are extensively used for the preservation of milk. Boron compounds can be detected in the following manner: Take not less than 10 grms. of the sample of milk and evaporate to dryness. Ignite the solid residue and add to the ash sufficient hydrochloric acid to render the whole slightly, yet distinctly, acid to litmus. Place a small slip of turmeric paper in the capsule in such a way that only part of it can be wetted, then evaporate to dryness at a temperature of 100°C . If that part of the turmeric paper placed in the liquid has become a definite brownish-red colour, owing to the formation of rosocyanin, boron compounds are present. A drop of caustic soda on the paper will produce a variety of colours, particularly green and purple, whereas hydrochloric acid will bring back the original red colour, which will change to green and blue on the addition of an excess of alkali.

It is not an easy matter to accurately determine the amount of boron compounds in milk, especially in a small quantity. R. T. Thompson ("Analyst," xviii. 184) observes that free boric acid may be titrated somewhat accurately by caustic alkali and phenol-phthalein, that is, if the liquid contains 30 per cent of glycerine. The neutral point then corresponds to the formation of NaBO_2 . Thus each c.c. of decinormal alkali required represents 0.0035 gm. of boric anhydride, B_2O_3 ; 0.0062 of crystallized boric acid, H_3BO_3 ; 0.00505 of anhydrous borax, $\text{Na}_2\text{B}_4\text{O}_7$; or 0.00955 gm. of crystallized borax $\text{Na}_2\text{B}_4\text{O}_7 + 10\text{H}_2\text{O}$. It is better to titrate the solution against a known weight of crystallized boric acid, than to assume the neutralizing power of the standard alkali to be correct: 0.310 gm. should neutralize 50 c.c. of decinormal alkali. When the foregoing method is applied to the determination of boric acid in milk, L. de Koningh recommends the addition of 1 c.c. of a strong solution of caustic soda to 10 grms. of the milk, evaporation of the liquid and ignition of the residue. The ash should then be boiled with water, the residue again ignited and again boiled with water. The two solutions will contain all the borates present. They should be mixed together, a drop or two of methyl-orange added, then decinormal sulphuric acid carefully dropped in until the liquid becomes slightly pink after stirring. After boiling the liquid for a minute or two to expel carbon dioxide, it is cooled and half its amount of glycerine added. A few drops of phenol-phthalein solution is next added and the liquid titrated with decinormal caustic soda until it becomes pink in colour. This method is more reliable for large amounts of boron compounds than for small quantities.

Another method for determining boron compounds in milk is to convert them into volatile methyl borate, then to decompose this compound after distilling with lime or other base. This method, apparently devised by Rosenbladt and Gooch, has been modified by Penfield and Sperry, Gilbert, Cassal, and Hehner, the following being the preferable method (C. E. Cassal, "Analyst," xv. 230; and O. Hehner, "Analyst," xvi. 141):—

Add to 50 grms. of cream or 100 grms. of milk caustic soda to render alkaline, evaporate to dryness and ignite the residue. Reduce the ash, not necessarily white, to a powder and transfer by means of a

little methyl alcohol and a few drops of water, to a conical flask of 200 to 300 c.c. capacity. Insert a caoutchouc stopper provided with a tapped funnel and delivery tube. Add acetic acid to make the contents of the flask acid, then add 5 c.c. of methyl alcohol. The flask should be connected with a condenser by means of a flexible joint (to allow the contents to be occasionally shaken). Place the liquid on an oil-bath, and distil almost to dryness; 5 c.c. of methyl alcohol is again added and the distillation again continued. Ten such treatments, with distillation, are quite sufficient, and in some cases even less suffice. Test the residue in the flask with turmeric paper to be certain that the boric acid has completely volatilized. Cassal recommends the addition of a few drops of water before distillation, as it helps greatly in the operation. He also suggests several distillations of small quantities in preference to once adding a large volume of methyl alcohol, better results being obtained. Mix the distillates which contain methyl borate, in a vessel containing a known weight of freshly burnt lime. The amount of boric acid can be calculated after evaporating to dryness and burning the residue, by observing the increase in weight. This is not the most satisfactory of methods as there are many difficulties and sources of error to contend with. Hehner prefers to use a solution of crystallized sodium phosphate instead of caustic lime. The methyl borate becomes decomposed after evaporating with this reagent. If boric acid is absent, the residue left after igniting will be solely sodium pyrophosphate $\text{Na}_4\text{P}_2\text{O}_7$, but if boric acid or methyl borate are present, the reaction brought about will form sodium metaphosphate and baborate $\text{Na}_4\text{P}_2\text{O}_7 + 2\text{B}_2\text{O}_3 = 2\text{NaPO}_3 + \text{Na}_2\text{B}_4\text{O}_7$.

Thus 0.133 gm. of sodium pyrophosphate produced by the ignition of 0.332 gm. of crystallized sodium phosphate $\text{Na}_2\text{HPO}_4 + 10\text{H}_2\text{O}$ will react with and fix 0.070 gm. of B_2O_3 , which represents 0.124 gm. of crystallized boric acid, or 0.191 gm. of crystallized borax. When performing the test it is preferable to use a solution of about 2 per cent strength of sodium phosphate instead of a solution of exactly known strength. Take e.g. 20 c.c. of this solution and add to the distillate containing methyl borate. Take an equal quantity of the same solution, measured with the same pipette, evaporate separately and ignite both residues. Weigh the two ignited residues and the difference in weight represents the B_2O_3 of the sample. Great caution must be exercised in heating the residue after evaporation, to prevent any loss, and ignition should be carried on in a covered platinum dish, the temperature being gradually raised until the residue fuses. O. Hehner prefers to collect the distillate containing methyl borate in a receiver containing caustic soda, evaporates, adds dilute mineral acid until absolutely neutral to methyl orange, then glycerine and phenol-phthalein, and lastly titrates with standard caustic soda to determine the boric acid. A. R. Tankard has proved by experiments that the results are the same as those obtained by evaporation of the distillate with sodium phosphate and weighing the ignited residue.

Cassal and Gerrans ("British Food Journal," 4, 210) describe the following process for the determination of boric acid:—

From 15 to 20 grms. of the sample, such as milk, is made distinctly

alkaline with a saturated solution of $\text{Ba}(\text{OH})_2$ in a platinum dish, evaporated to dryness, well charred, broken up, made just acid with HCl and extracted with successive quantities of hot water, the filtrates being mixed in a 100 c.c. flask. The filter and contents are transferred to the platinum dish, again made distinctly alkaline with $\text{Ba}(\text{OH})_2$, and carefully ignited. The ash is dissolved in a little HCl (1 : 3), the solution and washings added to the first solutions in the flask, and the whole made up to exactly 100 c.c. Ten c.c. of this ash solution is then pipetted on to 10 to 15 grms. of pure sand, the mixture is made alkaline with $\text{Ba}(\text{OH})_2$ solution and evaporated to dryness with occasional stirring. When dry it is made just acid with HCl (1 : 3), when 2 c.c. of saturated solution of oxalic acid and 2 c.c. of an alcoholic solution of curcumin (1 grm. per litre) are mixed in. The dish is then placed on a water bath, covered with a funnel, the stem of which is connected with a set of potash bulbs charged with $\text{Ba}(\text{OH})_2$ solution. Air is then gently aspirated through the apparatus until the mass in the dish is dry. An additional 1 c.c. of curcumin solution is then added, well mixed in, and the mass again dried. The dry mass is then extracted with small successive quantities of alcohol or methylated spirit, the solutions obtained being filtered into a flask. When the sand mixture is freed from colour the liquid in the potash bulbs is poured upon it and dried, care being taken that it is alkaline with $\text{Ba}(\text{OH})_2$. The mixture is treated as before with HCl , oxalic acid, and curcumin solutions, and the processes of evaporation and alcoholic extraction are repeated. The further yield of coloured alcohol is added to that it first obtained.

A standard colour is prepared by using 10 c.c. of boric acid solution (1 c.c. = 0.1 Mgm. B_2O_3) in precisely the same manner, the coloured alcoholic extracts being made up to 200 c.c. By comparing the depth of colour given by the ash solution extracts with this standard, the amount of boric acid in the quantity of solution operated on may be determined, and from this, the amount in the original sample calculated.

Formaldehyde is probably by far the most efficient of milk preservatives. It is usually employed in the form of a solution containing 40 per cent of formic aldehyde, $\text{H} \cdot \text{CHO}$. The following are useful methods for the detection of formic aldehyde in milk :—

S. Rideal ("Analyst," xx. 158) considers that Schiff's reagent is a delicate test for formaldehyde in milk, if the solution used is slightly acid. To prepare Schiff's reagent mix 40 c.c. of a 0.5 per cent solution of fuchsine with 250 c.c. of distilled water, add 10 c.c. of sodium bisulphite solution of 1.375 specific gravity, then 10 c.c. of pure strong sulphuric acid. Allow the mixture to stand until it becomes colourless. Another method is to add sufficient of a solution of sulphurous acid to take away the colour of the fuchsine solution. If too large a quantity of sulphurous acid is added it will be impossible to find traces of formaldehyde. This test is useful as a confirmatory reaction and can be applied to milk as described by Richmond and Boseley ("Analyst," xx. 155). Sulphuric acid is added in small quantities to precipitate the casein, the liquid filtered and a little of Schiff's reagent

is added to the filtrate. The amount of formaldehyde can be somewhat roughly estimated by the intensity of the red colour. No distillation is required in this test, which is an advantage. Richmond and Boseley found that aqueous solutions of milk sugar on the addition of Schiff's reagent give no coloration even when boiled with dilute sulphuric acid, though it has been stated that under some conditions, not defined, a red colour appears.

O. Hehner ("Analyst," *xxi.* 94) makes use of a test for detecting formalin in milk, the chief feature of which is the addition of strong sulphuric acid to the milk. If mere traces of formaldehyde are present, the liquid becomes a violet-blue colour. Richmond and Boseley apply the test by adding an equal measure of water to the milk and using sulphuric acid of 90 to 94 per cent strength. By using the test in this manner, its delicacy is considerably increased. If in 200,000 parts of milk there is one of formalin, a violet-blue colour will be produced at the junction of the two layers, which will remain permanent for some days. If formaldehyde is absent, a slight greenish colour may be observed, and lower down in the acid after some hours a brownish-red colour becomes noticeable. This, however, cannot be mistaken for the blue colour denoting the presence of formaldehyde. Hehner's reaction is simple and delicate. It is not produced by acetaldehyde. Richmond and Boseley state that it is not practicable with large quantities of formaldehyde—0.5 per cent would, for example, give no blue coloration. Richmond and Boseley attribute Hehner's reactions to the proteids of milk, as they find that egg-albumin and peptone give the reaction, whereas gelatine does not. Hehner, however, could not obtain a reaction with a solution of peptone, and only the very slightest response from egg-albumin, which he thought was due to some impurity rather than to the albumin itself. He was of the same opinion concerning the gelatine. N. Leonard ("Analyst," *xxi.* 157) states that Hehner's reaction can easily be obtained if commercial sulphuric acid is employed, but it fails altogether when pure redistilled acid is used. When ferric chloride or platinic chloride was mixed with pure sulphuric acid, it was found that the milk containing formaldehyde became violet-blue in colour. The pure acid contained no trace of iron whereas it was found that the commercial acid did. Leonard deduces from this, that a feeble oxidizing agent must be present for the production of Hehner's reaction. The addition of ferric chloride in considerable quantities does not improve the test. A trace of ferric chloride, however, renders the reaction more distinct. Hehner confirms this statement of Leonard.

O. Hehner ("Analyst," *xxi.* 94) describes another test which is only useful for testing the presence of a small amount of formaldehyde. This method is as follows: Add one drop of a dilute aqueous solution of phenol to the distillate from a sample of milk, or other substance. Pour the mixture upon some strong sulphuric acid contained in a test tube and it will be noticed that where they meet, a bright crimson colour appears if one part of formaldehyde in 200,000 be present. If there is a larger proportion present a milky-white zone above the crimson tinge appears. An orange-yellow colour denotes acetaldehyde.

This reaction will not be successful unless carried out exactly as described. It is important that only a trace of phenol should be used.

Trillat ("Compt. Rend." cxvi. 891) suggests the following test for formaldehyde. Mix the solution containing the formaldehyde with a solution of dimethylaniline in slight excess of sulphuric acid; shake well together. Heat the liquid for half an hour on the water bath, make alkaline and boil until there is no odour of dimethylaniline. Filter, and moisten the filter paper with acetic acid. A sprinkling of some powdered lead oxide will produce a blue colour if formaldehyde is present. The blue colour, which is not stable, is due to the formation of tetramethyl-diamido-diphenylmethane. Another test depends on the fact that a white precipitate is formed from a mixture of a solution of formaldehyde and 0.3 per cent solution of aniline. This precipitate is anhydro-formaldehyde-aniline. It can be weighed and the amount of formaldehyde present ascertained. Acetaldehyde also gives a precipitate. As the precipitate dissolves in hot water, and reappears on cooling, the test must be carried out in the cold. The precipitate given by acetaldehyde is more soluble than that given by formaldehyde. Trillat states that formaldehyde cannot always be detected in preserved foods after some lapse of time as condensation-products are formed. Richmond and Boseley are of the same opinion as Trillat, but they state that formaldehyde can always be detected in milk by this test unless the milk is curdled. If it be desired to weigh the precipitate of anhydro-formaldehyde-aniline it should be allowed to stand for forty eight hours, filtered off, dried at 40° and weighed. Its formula is $C_6H_5NCH_2$, and 100 parts are equivalent to 28.5 parts of formaldehyde.

Hydrochloric Acid Test.—Commercial hydrochloric acid (sp. gr. 1.2) containing 0.2 per cent of ferric chloride per litre is the reagent used. Add to 10 c.c. of milk, contained in a porcelain vessel, an equal quantity of the acid reagent. Slowly heat over a naked flame almost to boiling, shaking the vessel in order to break up the curd. A violet coloration denotes formaldehyde, varying in depth according to the amount present. If formaldehyde is absent the solution gradually turns brown. One part of formaldehyde in 250,000 parts of milk can easily be detected, before the milk turns sour, by this test. When this occurs the limit of delicacy is 1 part in 50,000. Various aldehydes in milk give colour reactions when subjected to the above treatment, but formaldehyde alone gives the violet coloration, which is perfectly easy to distinguish.

Confirmatory Tests with Distilled Milk.—To confirm the above tests distil 100 to 200 c.c. of the milk and use the first 20 c.c. of the distillate for testing purposes.

1. Add a drop of Schiff's reagent to a few drops of distillate in a test tube. If aldehyde is present a pink coloration will soon appear, deepening on standing.

2. Add a few drops of a 1 per cent aqueous solution of resorcin or phenol to 5 c.c. of the milk distillate. A crimson colour denotes formaldehyde and not other aldehydes.

3. To a small amount of milk distillate (slightly acidified with

sulphuric acid to fix any free ammonia before distillation) add a few drops of Nessler's reagent. Traces of formaldehyde give a yellow colour, whilst larger amounts produce a darker colour on standing, and forms a grey precipitate.

Determination of Formaldehyde in Milk.—The determination of formaldehyde is a matter of considerable difficulty when it is present in such minute amounts. Add 1 c.c. of 1:3 sulphuric acid to 100 c.c. of milk and distil in a 500 c.c. Kjeldahl flask. Use a low circular evaporating burner to avoid frothing. Smith states that the first 20 c.c. of the distillate or one-fifth of the original quantity contain almost one-third of the total formaldehyde. Collect 20 c.c. of the distillate and use the following potassium cyanide method for the determination of formaldehyde. To 10 c.c. of tenth-normal silver nitrate add 6 drops of 50 per cent nitric acid, using a 50 c.c. flask. Then add 10 c.c. of a solution of potassium cyanide containing 3.1 grms. of KCN in 500 c.c. of water making up to the 50 c.c. mark. Shake well, filter and titrate 25 c.c. of the filtrate with tenth-normal ammonium sulphocyanate using ferric chloride as an indicator. Take another portion of 10 c.c. of tenth-normal silver nitrate and acidify with nitric acid, add 10 c.c. of the potassium cyanide solution to which the above 20 c.c. of formaldehyde distillate has already been added. Make up the whole to 50 c.c., filter, and titrate 25 c.c. of the filtrate with decinormal ammonium sulphocyanate for the excess of silver as before.

To calculate the amount of potassium cyanide used up by the formaldehyde, in terms of decinormal ammonium sulphocyanate, multiply the difference between the two results by two, and the total amount of formaldehyde can be calculated by multiplying the amount found to be present in the 20 c.c. of distillate by three.

The formula $\text{CH}_2\text{O} + \text{KCN} = \text{KOCH}_2\text{CN}$ shows the reaction which takes place between the formaldehyde and the potassium cyanide, from which the formaldehyde is easily calculated.

When milk is preserved with formic aldehyde it usually contains .0002 to .006 per cent. The Government Laboratory consider .001 per cent as the maximum allowable, but owing to the fact that the Government chemists examine the milk late, much of the aldehyde may have disappeared. It must be estimated as quickly as possible, as micro-organisms destroy the aldehyde.

The following is a useful method :—

Blow a bulb of about 50 c.c. capacity on a piece of soft glass tubing with a quarter-inch bore. Draw out one end of the glass tube close to the bulb, into a capillary tube, and turn at right angles to the bulb. Turn the tube at the other side of the bulb at right angles to the bulb. Pour 10 c.c. of the milk sample into the bulb and make slightly acid, if the milk is not already sour. Seal up the end of the capillary tube and place the bulb in a paraffin bath taking care that it is completely immersed. Connect the open tube with a series of bulbs each containing 5 c.c. of water, a very short rubber connexion being used. Place the bulbs in a bath containing cold running water. Heat the paraffin to 120° C. and distil the milk almost to dryness. To pre-

vent frothing up the tube, warm it with a burner. As soon as the milk is nearly dry, break the capillary tube at the end, and pass a slow steady current of air through the apparatus by means of a water pump. Raise the temperature of the paraffin until it reaches 200°C . then keep it at that temperature for at least fifteen minutes. Disconnect the bulbs and test the second bulb for formic aldehyde. Unless the original solution contained a considerable amount, none will be found. If there is any present, determine the amount, then test the third bulb. Wash out the first bulb with distilled water, see that the liquid does not exceed 20 c.c., add two drops of litmus and a few drops of decinormal sodium carbonate solution to neutralize the free acid present. The formaldehyde can then be determined by the cyanide method above described.

In 1909 a report was made to the Local Government Board by Dr. J. M. Hamill on the "Use of Preservatives in Cream".

The use of preservatives is of importance in the cream-trade, and many convictions under the Sale of Food and Drugs Acts have been recorded when the amount of boron preservative used has been above that recommended by the 1901 Departmental Committee (0.25 per cent, expressed as boric acid), which added that boron preservative *only* should be employed. Dr. Hamill in his report first deals with the effect of boric acid on health, the general conclusion being that there is a preponderance of opinion that boron compounds cannot safely be regarded as incapable of exerting a deleterious action upon health. The use of cream "thickeners," such as gelatin, starch paste, and "suerate of lime," is commended to the analyst's attention; the last-named compound being openly sold by wholesalers for admixture with cream. In the section on the use of preservatives, Dr. Hamill remarks that "apart from preservative power the qualities especially requisite in such a preparation are ready solubility and freedom from any objectionable taste". Mixtures of boric acid and borax in such proportions as to produce a compound as nearly neutral as possible are the chief commercial preservatives. Most cream-preservatives contain, in addition, a small quantity of saccharine to mask incipient sourness. The recommendation of the 1901 Committee is sometimes infringed by the addition of sodium salicylate or sodium benzoate to boron preservatives, the makers relying upon the presence of these organic preservatives being overlooked when the boric-acid content is found to be within safe limits. Formalin has proved to be unsuitable as a cream-preservative. Dr. Hamill has been informed that sodium fluoride is used in some cases, and he refers to it as a "dangerous substance". The composition of cream-preservatives examined are represented in the table on page 68.

The directions for use are generally such as will ensure approximately the presence of 0.25 per cent of boric acid in the cream. Cream sold in jars or in small bulk generally contains preservatives, more being used in summer than in the cooler months. Imported cream practically always contains preservatives. Hydrogen peroxide differs from ordinary preservatives in that when properly used little or none of it remains in the cream. One firm who use it add 100 c.c. of

a 3 per cent solution to each gallon of freshly separated cream at 120° F., and maintain at that temperature for ninety minutes in a closed vessel. There "appears to be no doubt that it is impracticable to carry on a jug-cream trade . . . without the use of preservatives," and this trade, Dr. Hamill adds, is undoubtedly a convenience to the public. Experimental inquiry has substantiated the statement that 0.25 per

No.	Boric Acid (H_3BO_3).	Anhydrous Borax ($Na_2B_4O_7$).	Soluble Saccharin.	Other Sweetening or Preservative Substances.	Moisture, etc., by Difference.
	Per cent	Per cent	Per cent	Per cent	Per cent
1	84.32	15.15	Nil.	Nil.	0.53
2	54.96	20.85	0.85	19.39 (a)	3.95
3	76.76	22.12	1.18	Nil.	Nil.
4	77.98	21.53	Nil.	Nil.	0.49
5	77.13	22.22	Nil.	Nil.	0.65
6	73.02	24.24	Nil.	Nil.	2.74
7	75.66	22.61	0.93	Nil.	0.8
8	75.39	21.01	Nil.	0.59 (b)	3.01
9	59.56	32.09	Nil.	3.78 (c)	4.57

(a) Cane sugar, 17.45; salicylic acid, 1.94. (b) Cane sugar. (c) Sodium benzoate.

cent of boric acid is insufficient to preserve cream for more than three or four days at 71° F., but 0.4 per cent keeps it for four to seven days at this temperature. Dr. Arthur Harden's results are summarized in the following statements:—

Boric acid in the presence of an alkali (7 grms. of Na_2O per 100 grms. of boric acid) is a more efficient preservative than boric acid alone.

With this proportion of alkali 0.4 per cent of the acid is practically as effective as 0.5 per cent at 65° F., and slightly less at 71° F. Cream may be preserved by either of these proportions for about four to seven days at temperatures up to 71° F.

0.5 per cent of boric acid does not prevent development of moulds in cream after four to seven days.

The recommendations which Dr. Hamill submits to the Local Government Board are briefly as follows:—

1. Boric acid or mixtures with borax should be the only preservative allowed to be used in cream.

2. An exception might be made in respect of the use of hydrogen peroxide, providing that only traces are allowed to remain in the cream.

3. (a) Declaration of the presence of preservative to the purchaser, whether wholesale or retail, should in all cases be adequate, and preserved cream should be differentiated from cream containing no added preservative. (b) The maximum amount of boron preservative allowed, calculated as boric acid (H_3BO_3) should be 0.4 per cent from May to October inclusive, and 0.25 per cent during the remainder of the year.

(c) Cream containing preservatives should contain at least 40 per cent of milk-fat.

4. The presence of sweetening agents, such as saccharine, should be notified to purchaser.

In an addendum to the report, Mr. G. W. Monier-Williams, Ph.D., deals with the detection of small quantities of benzoic acid, salicylic acid, and saccharine in cream. His process is as follows:—

Concentrated phosphoric acid (1 c.c.) is added to 100 grms. of cream, and the mixture heated with constant stirring on an asbestos gauze over a Bunsen burner until all the water has been driven off (mere traces only of benzoic and salicylic acids volatilize owing to their great solubility in butter-fat). The temperature should not rise above 120° C. The clear fat is filtered through a dry filter, cooled to 60° to 70° C. and shaken out with 50 c.c. of sodium-bicarbonate solution (0.5 per cent) heated to the same temperature. The separated alkaline liquid is filtered, acidified with strong hydrochloric acid (1 c.c.), cooled, and extracted with three successive quantities (15 to 20 c.c.) of ether. The combined ethereal solutions are dried with calcium chloride, and the ether distilled off.

If saccharine is present the residue will taste distinctly sweet. To detect the presence of salicylic or benzoic acid, strong ammonia (1 c.c.) is added to the residue, which is then evaporated to dryness and taken up with four drops of water, and a minute drop of a 10 per cent solution of iron alum added. The characteristic purple coloration or buff precipitate will indicate the presence of salicylic acid or benzoic acid respectively. The limits of the test are benzoic acid 0.0075 per cent, saccharine 0.001 per cent, salicylic acid 0.0002 per cent.

Cream.—Cream is the thick fatty layer which rises to the surface of milk when it is allowed to stand, or is otherwise induced to separate, leaving the skimmed milk (or separated milk when a centrifugal process is used). There is no legal standard for cream, other than the necessity, of course, that it shall not be adulterated; but the best cream contains 50 per cent of fat and no good cream should contain less than 30 per cent. The table on page 70 shows the composition of a number of samples separated by the Aylesbury Dairy Company, the analyses being those of Vieth and Droop Richmond.

The principal adulterants of cream, are, as stated above, gelatine, starch, and sucrate of lime.

Starch may be detected by means of iodine in the usual manner.

Gelatine can be detected by the method proposed by Stokes.

An acid solution of mercuric nitrate is prepared by dissolving mercury in twice its weight of nitric acid of 1.42 specific gravity and diluting this to twenty-five times its volume, with water; 10 c.c. of the milk or cream is mixed with an equal volume of this solution, the mixture shaken and 20 c.c. of water added. The liquid is again shaken and allowed to stand for five minutes and then filtered. If much gelatine be present the filtrate will be opalescent and cannot be obtained clear. To a portion of the filtrate add an equal volume of saturated solution of picric acid. In the presence of gelatine a turbidity or yellow precipitate, according to the amount present, will be formed.

	Fat.			Solids not Fat.		
	Average.	Highest.	Lowest.	Average.	Highest.	Lowest.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1883	35.5	41.1	31.8	6.8	7.1	6.3
1884	35.3	39.0	32.6	6.8	7.0	6.4
1885	42.5	51.1	35.9			
1886	44.25	46.0	41.5			
1887	43.2	46.1	40.6			
1888	45.55	48.0	43.5			
1889	47.35	49.9	45.5			
1890	48.35	50.5	45.3			
1891	49.05	51.9	45.7			
1892	46.85	49.5	43.9			
1893	47.7	50.9	45.0			
1894	49.0	51.2	46.5			
1895	49.1	50.6	47.4			

To detect sucrose or calcium succate, the process of Rothenfusser ("Zeit. Untersuch. Nahr. Genuss.," 1910, xix. 465) may be used. A portion of the sample is heated to 90° C., and then treated with an equal volume of a mixture of 2 volumes of a solution of lead acetate (500 grms. in 1200 c.c. of water) and 1 volume of ammonia solution of specific gravity 0.944. The whole is well shaken for thirty minutes and after a minute is filtered; 3 c.c. of the filtrate is mixed with 3 c.c. of a solution of diphenylamine (2 grms. of diphenylamine in 10 c.c. of alcohol, 25 c.c. of glacial acetic acid and 65 c.c. of hydrochloric acid), and heated in the water bath for five to ten minutes. Another portion is tested with Fehling's solution to see if all the lactose has been removed. If no reduction takes place in the latter test, a blue coloration in the former is proof of the presence of sucrose.

In the detection of calcium succate in cream, the mixture of cream and ammoniacal lead acetate should be heated to 65° and should preferably be allowed to stand some minutes before filtering.

Salicylic may be extracted from the milk which has first been treated with acid nitrate of mercury solution (see under determination of lactose), by ether and the ether extract tested with ferric chloride solution, when the characteristic violet colour will be obtained. Carbonate of soda will be shown by the effervescence of the ash when treated with hydrochloric acid.

Added Colouring Matter in Milk.—Annatto has practically been the only colouring matter used until recently. Milk dealers have considered caramel unsuitable, as it has too much brown and too little yellow in its composition, and therefore it is difficult to imitate the natural colour of milk. Annatto, on the contrary, gives a rich, creamy appearance to the milk, even if watered, when carefully used with the right dilution. This accounts for its popularity with milk dealers. One or more of the azo-dyes have been much used of late, as they give just as good a cream colour as annatto.

Appearance of Artificially Coloured Milk.—The natural colour of milk is to be found chiefly in the cream. Artificial colouring, on the other hand, spreads through the whole of the milk. When the cream has risen to the surface, the underlying milk, instead of having the bluish colour characteristic of skimmed milk, is the same colour as the cream, especially if much colouring matter has been used. An analyst in examining a number of samples can often judge artificially coloured milks from their appearance alone.

Nature of Annatto.—Annatto, arnatto, or annotto is a reddish-yellow colouring matter, which is derived from the pulp enclosing the seeds of *Bixa Orellana*, a shrub grown in South America and the West Indies. The form used in the coloration of milk is a solution of the colouring matter in weak alkali (see p. 247).

Nature of "Anilin Orange".—The azo-dyes are the best of the coal-tar colours for colouring milk, and they are, therefore, most used. The Department of Food and Drug Inspection of the Massachusetts Board of Health have had some samples of these commercial "milk improvers" analysed, and they have found them to be mixtures of two or more members of the diazo-compounds of anilin. A mixture of what is known to milk dealers as "Orange G" and "Fast Yellow" gives exactly the same colour as one of these preparations which was obtained from a milk dealer who had previously used it. A generic name, such as "a coal-tar dye" or "anilin orange" is more convenient for purposes of prosecution or otherwise, than a particular description, considering our present knowledge of the subject.

Systematic Examination of Milk for Colour.—Leach employs the following general method for examining suspected milk samples—Curdle about 150 c.c. of the milk by means of heat and acetic acid, in a porcelain basin over a Bunsen flame. Gather the curd into one mass by aid of a stirring rod, when it is easy to pour off the whey. If, however, the curd is too finely divided in the whey, strain it through a sieve or colander. All of the annatto, coal-tar dye, and part of the caramel present in the milk will be found in the curd. Place the curd, which should be free from all whey, in a corked flask with ether and allow it to digest with it for several hours until the fat has been extracted and with it the annatto. Then pour off the ether, and if the curd is perfectly white, either the milk is not coloured, or annatto has been used. If, on the contrary, the curd is coloured more or less deeply, anilin orange or caramel has been used, the amount being roughly estimated by the depth of the colour. Hence it is obvious that of the three colours annatto, caramel, and anilin orange, only annatto can be extracted by ether. The curd will have a brown colour if caramel had been present and a brightish orange if anilin orange has been used. The following tests should then be applied:—

(a) *Tests for Annatto.*—Evaporate the ether extract containing the fat and annatto, if present, on a water-bath. Make the residue alkaline with sodium hydroxide, pour upon a small wet filter, which will keep back the fat, and allow the annatto, if present, to permeate the pores of the filter as the filtrate passes through. After washing off the fat carefully under the water tap, it will be found that all the annatto of

the milk used for the test has collected on the filter, giving it an orange colour, which is fairly permanent and varying in depth according to the amount of annatto present. To confirm this test add stannous chloride to the coloured filter, when the characteristic pink colour is produced.

(b) *Tests for Caramel.*—Take the curd after the ether has been poured off, and after it is free from fat, place in a test-tube and shake well with hydrochloric acid. If caramel is present, this acid solution gradually turns a deep blue on shaking. The white fat-free curd of uncoloured milk would show the same colour if all the fat has been thoroughly extracted from the curd. It is most necessary for the quick formation of the colour that the curd should be entirely free from fat. The reaction will be quicker if gentle heat is applied. Caramel is only indicated when the blue coloration of the acid appears in conjunction with a coloured curd. The coloration is of a brownish-blue when much caramel is present. Even if there is a blue coloration it is a good plan to confirm its presence by testing a separate portion of milk as follows:—

Curdle about a gill of milk by adding strong alcohol. Filter off the whey, and add a small quantity of subacetate of lead. Collect the precipitate thus formed upon a small filter and dry in a room free from hydrogen sulphide. Pure milk treated in this manner gives a residue wholly white or at the most a very pale straw colour, but, if caramel is present, the residue is a more or less dark-brown colour varying according to the amount present.

(c) *Tests for Coal-tar Dye.*—When azo-dye has been used to colour the milk, apply strong hydrochloric acid to the coloured curd in a test tube and the liquid will immediately turn pink. If much anilin dye has been added to the milk, the curd will sometimes have a pink coloration when hydrochloric acid is applied directly to it, before ether is added. The colour reaction with fat-free curd is unmistakable, and very delicate.

Lythgoe states that the presence of anilin orange in milk can be determined by directly adding 10 c.c. of strong hydrochloric acid to an equal quantity of the sample, mix well together and if the dye is present in more than minute traces, it will produce a pink coloration.

CONDENSED MILK.

The evaporation of water from milk, especially in Switzerland, and packing the condensed product and exporting it to other countries is now a very common and lucrative practice. The term condensed milk is usually applied to the thick syrupy product containing 40 to 50 per cent of water, but recently powdered milk has become a regular commercial article. Of powdered milk there is little to be said, since it is obvious that its composition should correspond with that of milk minus its water. The composition, to come within the regulation of the Board of Agriculture for milk, would have to be (on the basis of 11·5 per cent solids and 3 per cent fat)

Fat = 26 per cent
Non-fatty solids = 72 per cent.

The author has examined many samples and found the fat varies in the best brands from 26 to 30 per cent. The further composition of this preparation is indicated by the figures given under milk. The usual liquid condensed milk is sold as either unsweetened or sweetened, the latter containing added cane sugar and having much better keeping properties when exposed to the air, than the unsweetened variety.

The following represent the composition of the better-class condensed milks, and it is to be noted that there exists no legal standard for condensed milk so far as the amount of condensation is concerned, but of course the proper proportion of the various constituents of the milk, other than the water, must be preserved; skimmed milk when condensed may not be sold as condensed milk, without disclosing the fact that it is made from skimmed milk.

UNSWEETENED CONDENSED MILK.

Total Solids.	Fat.	Proteids.	Lactose.	Ash.	Observers.
51·61	15·67	17·81	15·40	2·53	Percy
36·10	11·06	12·75	—	—	Allen
43·00	9·8	11·3	18·5	2·5	Pearmain and Moor
23·10	8·10	8·66	—	1·55	Leach
39·80	12·21	13·10	—	2·4	Parry

SWEETENED CONDENSED MILK.

Solids.	Fat.	Proteids.	Lactose.	Cane Sugar.	Ash.	Observer.
68·10	11·05	10·95	—	—	—	Allen
74·40	10·8	8·8	16·0	37·1	1·7	Pearmain and Moor
74·29	10·65	8·46	11·97	41·92	1·29	Leach
73·11	11·61	9·1	13·0	38·00	1·4	Parry

Condensed unsweetened milks made from skim milk will show a low fat value, whilst the proteids and sugar will be high. The same is true, of course, for sweetened varieties.

It may be noted that in the United States an official standard exists for condensed milk. Standard condensed milk must contain at least 28 per cent of milk solids, 27·5 per cent of which is milk fat.

Analysis of Condensed Milk.—10 grms. of the well-mixed sample should be diluted to 100 c.c. with water. 10 c.c. of this mixture will then represent 1 gm. of the sample.

Total Solids.—10 c.c. of this (1 gm. of sample) are heated in a flat-bottomed capsule on the water bath and then transferred to the water oven. Five to six hours are required for complete desiccation. After weighing the total solids, these are ignited at a dull red heat and the ash weighed. Where much sugar is present, it is necessary to dry

the liquid on a little recently ignited asbestos, which is weighed with the dish itself.

Fat may be approximately determined by extraction with ether of paper saturated with 10 c.c. of the above solution (Adams' process) as described above; the Werner-Schmidt and centrifugal processes are not very suitable unless modified, especially when sweetened milks are in question. Leach ("Journ. Amer. Chem. Soc." 1900, 589) prefers the following method: 15 c.c. of a solution of 40 grms. of the sample made up to 100 c.c. (i.e. 6 grms. of the sample) are measured into a Babcock bottle, and 4 c.c. of a solution of copper sulphate (of the same strength as Fehling's solution) are added. The mixture is well shaken and the proteid precipitate and fat are rapidly separated by whirling in the centrifugal machine. The supernatant liquid containing the sugar is drawn off with a pipette, and the precipitate is twice washed with water, and the washings drawn off and added to the clear liquid. Water is now added to the precipitate up to 17.6 c.c. and 17.5 c.c. of sulphuric acid are added, and the liquid is then placed in the centrifugal machine and the reading of fat multiplied by three to give the percentage of fat (the bottles being graduated in reference to 18 grms. of the sample).

Proteins may be determined on 5 c.c. of the solution of 40 grms. in 100 c.c. This is diluted to 40 c.c., and copper sulphate solution added carefully until no further precipitation takes place, avoiding much excess of copper. Add a little very dilute caustic soda solution, leaving the solution faintly acid. Filter through a tared filter paper, wash, dry at 100 c.c. and weigh. Burn the paper, and take the difference between the weights of the precipitate and the ash as the proteids and fat. This, minus the amount of fat, gives the proteins.

Lactose may be determined in the filtrate and washings from the above process. These are made up to 100 c.c. with water and 10 c.c. of Fehling's solution are reduced by this liquid in the usual manner. The lactose may be calculated from the following formula:—

$$L = \frac{100 \times 0.067}{S \times 0.02}$$

where L is the percentage of lactose in the sample and S is the number of c.c. of milk solution of the above strength required to reduce the 10 c.c. of Fehling's solution.

Cane sugar, in sweetened condensed milk, may be taken as the difference figure after milk sugar, proteids, fat and ash have been determined.

Polarimetric determinations are somewhat unreliable with condensed milks, since the heat to which the milk has been exposed during evaporation causes changes which cannot be allowed for. The following methods are approximately accurate:—

Stokes and Bodmer ("Analyst," x. 10) add 1 per cent of citric acid to coagulate the milk without heating, dilute, filter and determine the reducing power of the clear filtrate. One per cent of citric acid is added to another portion of the filtrate, and the authors give ten minutes as the length of time for boiling the solution, though, accord-

ing to Watts and Tempany ("Analyst," xxx. 119) it is better to boil for at least thirty minutes. The solution is allowed to cool, is neutralized, and the reducing power again determined. The invert sugar formed from the sucrose is measured by the difference in the two reductions.

Leffmann and Beam use invertase for inversion. They precipitate the proteins with mercuric nitrate and polarize the clear whey. The acid is carefully neutralized in a portion of the filtrate, one drop of acetic acid is added, also a small quantity of invertase and a few drops of an antiseptic. The whole is incubated for twenty-four hours at 35° to 40°, and the liquid made up to known quantity and polarized again. The difference between the two readings is due to inverted sucrose.

Bigelow and McElroy ("Jour. Amer. Chem. Soc.," 1893, 15) suggest the following method for determining sugars, in condensed milk. The reagents used are acid mercuric iodide and alumina cream.

Place the entire contents of a can in a porcelain dish and mix thoroughly. Weigh a number of portions of 26.048 grms. into 100 c.c. flasks. Add water to two of the portions and boil the solutions. Boiling is necessary in order to ensure normal rotations. To one portion add a few c.c. of a solution of 53 grms. of potassium iodide, 22 grms. of mercuric chloride and 30 c.c. of glacial acetic acid in 1000 c.c. of water; and also a little alumina cream. Make up to 100 c.c. and filter. The polarimetric reading of the filtrate is determined. Heat the other weighed portion in the water-bath to 55°, add one-half of a cake of compressed yeast, keeping the temperature at 55° for five hours. Clarify the solutions as before, cool to room temperature, make up to 100 c.c., mix, filter and take the polarimetric reading. The amount of

cane sugar can be determined by the formula— $C = \frac{100 D}{142.66 - t/2}$, where C is the percentage of sucrose, D is the difference between the direct and inverted readings, and t is the temperature.

Determine the total reducing sugar by one of the reducing processes on a weighed portion of the original material; if the sum of it and the amount of cane sugar determined by the inversion method is equal to that obtained by the direct reading of both sugars before inversion, no invert sugar is present. If the amount of reducing sugar seems excessive, milk sugar may be separated as follows:—

Dissolve 25 grms. in water, boil the solution, cool to 80°, add a solution of about 4 grms. of glacial phosphoric acid, keep the mixture at 80° for some minutes, then cool to room temperature, make up to a known volume, mix and filter. Next, add potassium iodide in such quantity as not to quite neutralize the acid, and water sufficient to make up for the solids precipitated by the acid. Filter the mixture, and measure the filtrate in portions of 100 c.c. into 200 c.c. flasks. Add a solution containing 20 mgs. of potassium fluoride and half a cake of compressed yeast to each flask, then allow the mixture to stand for ten days at a temperature of from 25° to 30°. Fermentation will remove the invert sugar and cane sugar while the milk sugar is still

unaffected. Fill the flasks to the mark, shake, and determine the milk sugar by both reduction and the polariscope.

The amount of copper reduced by the milk sugar and invert sugar in the original sample less the milk sugar remaining after fermentation is due to invert sugar. C. B. Cochran ("Jour. Amer. Chem. Soc." 1907, **29**, 545-56) advises Wiley's acid mercuric-nitrate solution to invert sucrose in analysing sweetened condensed milk. He has found that this inverts sucrose only very slowly at temperatures below 15°; 50 c.c. of the solution to be inverted (containing 3 c.c. of mercuric solution per 100 c.c.) are polarized immediately the solution has been mixed at 15°, then heated in boiling water for 7 minutes then polarized again. The following formula gives the sucrose content in the case of normal solutions:—

Sucrose = $\frac{100 D}{142.66 - 0.5t}$ where D represents the difference in polarization before and after inversion and t the temperature. Leff-

mann employs the sesame oil test for detecting sucrose in condensed milk or milk sugar, i.e. 1 c.c. of sesame oil, 1 c.c. of concentrated hydrochloric acid and 0.5 grms. of the sample are well mixed together by shaking. The characteristic crimson coloration will be apparent within half an hour. This test is perfectly reliable and better than that to be found in the United States Pharmacopœia which depends on carbonization of the sucrose by strong sulphuric acid. A quick test for determining sucrose in milk and cream is to boil a mixture of 15 c.c. of milk, 0.1 gm. of resorcinol, and 1 c.c. of concentrated hydrochloric acid. Pure milk remains unchanged while sucrose gives a fine red coloration.

Baker and Hutton ("Analyst," xxxv. 512) have shown that the most accurate results, when a biological process is used, are obtained by using 0.5 gm. of washed brewer's yeast per 100 c.c. of a 2 to 3 per cent solution, and fermenting for 60 to 70 hours at 27°. Results varying from 90 to 100 per cent of the theoretical are obtained with lactose in the presence of dextrose, sucrose, maltose or invert sugar, the determination being made by a direct titration with Fehling's solution.

The Interpretation of Results.—In order to decide whether the milk from which the sample was prepared was genuine or not, an arbitrary standard must be agreed upon. Considering the Board of Agriculture regulations, it is necessary to adopt their minimum limits for this purpose. The fat value will be 3.0 per cent and the non-fatty solids 8.5 per cent. In the case of unsweetened condensed milks the fat of the original milk may be calculated from the formula:—

$$\frac{\text{Fat of original milk}}{\text{Fat of sample}} = \frac{8.5}{\text{Non-fatty solids of sample}}$$

Similar equations will give the ash of the original milk, and the total solids will be approximately obtained by adding 8.5 to the fat value.

In sweetened condensed milk, the fat and total solids in the sample should first be calculated to the basis of the condensed milk less the cane

sugar, that is by multiplying by $\frac{100}{100 - P}$ when P is the amount of cane sugar.

Now subtract the fat from the total solids (corrected as above), the result being the non-fatty solids calculated to the sugar-free condensed milk. This value divided by 8.5 (adopting the official minimum) will give the number of times the milk has been condensed.

The percentage of fat in the cane-sugar-free sample (corrected as above) divided by the number of times condensed gives the amount of fat in the original milk.

Approximately accurate results are also obtained by dividing the ash of the sample (corrected as above, in sweetened milks) by 0.7, the average ash value for normal milks. This gives the number of times the milk has been condensed, and the fat value of the sample (corrected) divided by this gives the fat value of the original milk.

The Analysis of Altered Milk.—If only a slight amount of acidity has developed in milk, no appreciable differences are noted between the analyses of such a sample, and of the unaltered milk. But if decomposition has gone so far that it is not possible to obtain a uniform emulsion, no analysis will give satisfactory results. If the sample can be well emulsified by the use of an egg beater, the analysis presents no difficulty. The following process is employed officially in the government laboratories when milk is very sour :—

From 10 to 12 grms. are weighed into flat platinum capsules and neutralized with decinormal strontia solution using phenol-phthalein as indicator. The liquid is then evaporated in a water bath until it has a nearly solid consistency, and whilst hot enough to keep the fat melted, 20 c.c. of dry ether (0.720) are poured on to the solids which are well stirred with a glass rod. The ether is filtered through a dry filter (10 cms. diameter) into a wide-mouthed weighing bottle. The solids are similarly treated with eight successive portions of 10 c.c. each, of ether. The filter paper is well washed with boiling ether, and any increase in its weight when dried is added to the weight of the non-fatty solids. These are dried in a water oven to constant weight, and the fat is weighed after evaporation of the ether. A deduction of 0.0042 specific gravity each c.c. of the strontia solution used, is necessary..

Allen recommends the examination of the whey when milk has curdled but not undergone any further change. He finds that the specific gravity of the whey varies in pure milks from 1.029 to 1.031 very rarely falling a little outside these limits. The solid matter in the whey of pure milk varies from 6.7 to 7.1 grms. per 100 c.c. So that a lower specific gravity or solid residue indicates watering.

Fat may be conveniently estimated in sour milk by the Werner-Schmidt process (p. 50).

Allen points out that the alteration in certain of the solids of milk on keeping makes it inadvisable to base an opinion alone or chiefly as to the genuineness of the milk on the amount of non-fatty solids. He prefers to determine the nitrogen and the ash, since any nitrogenous matter present will not, on decomposition, evolve nitrogen, so

that the determination of the nitrogen value will not be altered by decomposition within certain limits.

The value 0.5 per cent of nitrogen may be safely taken as the lowest permissible limit for nitrogen in genuine milk, as determined by the Kjeldahl process. In the same way adulteration may be presumed if the ash falls below 0.7 per cent, and not more than 30 to 33 per cent of this should be soluble in water.

When it appears necessary to determine the nature and amount of the loss in the non-fatty solids of a milk as a result of decomposition by keeping, it is necessary to estimate the amount of alcohol and volatile acids formed. The slight decomposition of nitrogenous matter with the evolution of a trace of ammonia is of very little importance.

The alcohol is estimated by distillation in the usual manner, the specific gravity of the distillate made up to the original bulk, giving the amount of alcohol. In distilling the milk, the free acidity should be first determined and the portion used for distillation first treated with half the amount of alkali necessary to neutralize it. If more completely neutralized, there is a risk of ammonia distilling over. The first 20 per cent of the distillate usually contains a little free acid, so that it should be kept separate, and then redistilled after complete neutralization.

The amount of proof spirit (see table) by volume, multiplied by 0.842, gives the weight of lactose which has undergone fermentation.

The amount of lactose lost by conversion into acetic acid is calculated by determining the total acidity and the acidity of the fixed residue of the milk. If 10 grms. be used, the number of c.c. of decinormal NaOH required by the volatile acids, is multiplied by 0.006 (acetic acid has a molecular weight of 60) and by 10. This gives the percentage of acetic acid, which, when multiplied by 0.425 gives the amount of lactose converted into acetic acid.

During the past few years, the use of soured milk as an article of diet has come much into vogue, and although it differs but little in chemical composition from ordinary milk apart from the presence of a larger quantity of lactic acid, its examination from a bacteriological point of view is sometimes required. The following interesting account of the souring of milk is due to F. W. Gamble (*"Pharm. Journ."* 1909, 1, 253).

In natural milk caseinogen and fat are very closely associated; a large proportion of the calcium and of the phosphoric acid present is also in more or less intimate association with the caseinogen. Caseinogen is coagulated by rennet and some bacterial ferments with conversion into casein. When milk is coagulated by rennet, the casein and fat together form a curd, separating from the whey, which contains in solution lactalbumen, lactose, salts, and a small quantity of whey-proteid formed as a decomposition-product of caseinogen. Rennet coagulates milk only in the presence of calcium salts; if these be completely removed by potassium oxalate before adding rennet coagulation does not occur. Milk that has been boiled is not coagulated by rennet, probably because the calcium salts are rendered insoluble; if they be partially removed from solution by the addition of

sodium citrate, the milk—known as “citrated” milk—is rendered less amenable to the coagulating action of rennet, and advantage is frequently taken of this means of preventing the formation of an indigestible solid curd by the action of the rennet of the gastric juice in the stomach of infants and invalids. Acids—such as lactic or acetic acid—precipitate caseinogen from boiled or unboiled milk; the calcium salts are removed from their natural combination and pass into solution.

Lactose, the principal carbohydrate of milk, is a disaccharide; as such it is not assimilable, but must undergo inversion before or during absorption. It is not readily inverted by ordinary yeasts, and is, therefore, not very susceptible to alcoholic fermentation. It is, however, very readily decomposed by a group of micro-organisms which by a hydrating process convert a molecule of lactose into four molecules of lactic acid, and are hence classed as “lactic acid bacilli”.

Milk is an almost ideal culture medium for both saprophytic and pathogenic bacteria, since it presents in an alkaline or neutral liquid proteid matter, carbohydrate, and salts, which, together, constitute a complete bacterial diet. Unless drawn from the cow under the most aseptic conditions, milk is immediately infected by a host of micro-organisms, derived from the teats of the cow, from the byre, the milker, and other sources. In so suitable a nidus very rapid multiplication of these bacteria ensues, so that milk drawn in what may be called strictly sanitary conditions, cooled to 45° F., and kept at that temperature, may contain an average of 4000 to 6000 bacteria per 1 c.c. after five hours, whilst London milk, as offered for sale, may contain from 1,000,000 to 4,000,000 bacteria per 1 c.c. The actual number of bacteria present in milk per 1 c.c. is, therefore, seen to vary very greatly, the determining factors being chiefly the conditions under which the milk is drawn, the temperature at which it is stored, and the length of time that elapses before examination. A characteristic rise and fall in the numbers has, however, been shown to take place. At about four hours after milking the number of saprophytic or putrefactive organisms has reached an initial maximum, and a fall in the total number of bacteria present is then noticed. This is due to the gradual multiplication of lactic acid producing organisms, which, by rendering the medium acid in reaction, inhibit the development of ordinary putrefactive bacteria, and ultimately procure their extinction. The total number of bacteria present then rises again to a second maximum many times greater than the first, the organisms now consisting almost wholly of lactic acid producing species, or those whose vitality has withstood the action of the acid produced. At this stage, either owing to exhaustion of pabulum or to the degree of acidity reached, growth is again checked, the lactic bacteria rapidly die out, and only moulds flourish.

Pasteur was the first to describe an organism characteristic of lactic fermentation and to demonstrate the distinction between this and alcoholic fermentation. Lister subsequently obtained from sour milk a bacterium which he grew in pure culture, and termed *Bacterium lactis*. In 1884 Hüppe grew the same organism on the then newly

introduced solid media, and termed it *Bacillus acidi lactici*, a name now applied to the whole family of micro-organisms possessing similar bacteriological properties and capable of decomposing lactose with formation of lactic acid. Many different species of lactic acid producing organisms have since been described and the same species have been described under different names, so that considerable confusion exists in the terminology of the subject. The different species exhibit small cultural variations, some growing well in the presence of oxygen, others better in deep vessels. They also vary in size and shape and in the type of lactic acid produced. Three organisms only need be described in any detail.

Ordinary lactic fermentation in this country is due chiefly to the bacillus of Hüppe, which is a non-motile oval rod 0.6 to 2 microns long. It is non-spore-bearing, and grows well at room temperature. It forms acetic and optically inactive lactic acids, and produces a solid curd separating from a clear fluid. Accompanying the Hüppe bacillus in sour milk, there is frequently found another non-motile organism in the form of short, thick rods = 1 micron long, called Günther's bacillus, or *Bacillus acidi paralactici*. It coagulates milk, producing as a result of the decomposition of lactose, dextro-rotatory or paralactic acid. These two organisms possess comparatively low vitality, are destroyed as the proportion of lactic acid increases, and are considered useless as therapeutic agents. In some parts of Europe a native lactic organism is found which differs considerably both from the bacillus of Hüppe and from that of Günther. This, known as *Bacterium Caucasium*, the Bulgarian bacillus, Massol's, or Boucard's bacillus, is very much larger than other lactic bacilli, is slightly motile, and produces lactic acid in abundance. It grows very slowly at room temperature, but freely at its optimum temperature of 100° to 105° F. It is possessed of great vitality, and withstands the action of its autogenous lactic acid to a higher degree than any other lactic acid producing organism yet discovered.

Milk that has been allowed to become sour is preferred by many people to milk in its natural sweet condition. Spontaneously soured milk contains fewer saprophytic bacteria than sweet milk a few hours old. The casein undergoes a slight degree of peptonization as a result of the fermentative process, and its partial precipitation by the lactic acid prevents the formation of a solid curd in the stomach. Besides being less cloying to the palate, sour milk is, therefore, also more digestible; amongst many country folk in Great Britain it is a regular article of diet, and especially so in Scotland. Buttermilk also is largely consumed; it contains but a small proportion of fat, is distinctly acid from the presence of lactic acid, and its proteids are present in a finely flocculent form. In many countries a doubly fermented milk is preferred to that which has undergone simple lactic fermentation. Milk does not readily undergo simple alcoholic fermentation; certain forms of yeast are, however, found to be symbiotic with the Caucasian bacillus, and these set up jointly a mixed alcoholic and lactic fermentation. The best-known examples of this double fermentation are seen in the preparations koumiss, kephir, yoghourt, matzoon, and leben.

Koumiss, as prepared by the nomadic Tartars of Russia, is made from mare's milk, the cultures being carried on by adding a small proportion of old fermented milk to the newly drawn milk. Koumiss contains about 1.7 per cent of alcohol and under 1 per cent of lactic acid. Kephir is prepared in the Caucasus from the milk of goats, sheep, or cows. Some old kephir is used to carry on the cultivation, or a few kephir grains are soaked in warm water, and when swollen and soft are added to new milk; fermentation takes from one to three days, according to the temperature, and the product remains good for a considerable time. It contains about 2 per cent of alcohol. Kephir grains are the dried scrapings from old vessels in which repeated fermentations have taken place: they are yellowish-brown in colour, irregular in size and shape, and have a characteristic odour resembling that of peptone, of which they contain a considerable proportion. Stored in a dry place, kephir grains retain their activity for many years; they contain, in addition to the yeasts and lactic acid producing bacteria, one or two forms of streptococci. Yoghourt prepared from maya ferment possesses properties resembling those of kephir, and is a staple food of the Bulgarians and other Balkan races. The peoples consuming these fermented milks as daily articles of diet are amongst the most healthy and long-lived races of mankind.

Many other applications of lactic fermentation to the preservation of foods might be quoted to show that the process is a general protection against putrefaction. The ubiquity of the bacilli of lactic fermentation ensures their ingestion with food-stuffs of all kinds, and their effect in the intestinal tube is, so long as they survive the changed conditions, exactly comparable with what is observed outside the body.

Lactic acid bacilli are a prominent feature of the normal flora of the small intestine; in this portion of the alimentary tract the alkaline intestinal secretions are rendered acid in reaction by the gastric juice and by the lactic, acetic, and other acids which are produced by the fermentative processes carried on in the abundance of material poured out from the stomach. In consequence of this acidity, and whilst it is maintained, anaerobic putrefactive bacteria gain no position in the small intestine. These proteolytic bacteria can exist only in a distinctly alkaline medium, and so long as a sufficient quantity of acid is produced, putrefaction does not occur. In the normal state the same condition of affairs should exist in the large intestine. In the normal colon and appendix aerobic bacteria and facultative anaerobes (such as the *Bacillus coli communis*) are found, but no such strictly anaerobic organisms as are present in pathological conditions. In the normal state, such anaerobic organisms as are ingested with the food are destroyed by the aerobic bacilli present. On the other hand, in enteritis, appendicitis, and the conditions associated with auto-intoxication the flora of the large intestine is characterized by a diminution in the number of aerobic organisms, the bacteria inhibiting putrefaction, and by an increase in the number of anaerobic micro-organisms which are the bacteria giving rise to putrefaction. These latter, by their growth in the albuminous intestinal contents, produce

soluble poisons which are absorbed into the circulation. Escaping destruction by the liver or other defensive mechanisms of the body, these toxins are considered to be active agents in the production of many forms of ill-health and chronic disease.

To Metchnikoff must be attributed the idea of changing the balance of power between these opposing forces of bacteria by the administration, under proper conditions, of a culture of living and highly active lactic acid producing bacteria. It should not, however, be supposed that nothing more than the administration of suitable organisms is necessary; they must be assisted by the concurrent use of a proper diet, calculated not only to favour the multiplication of lactic organisms but also to inhibit the growth of putrefactive bacteria. This is attained by the use of a farinaceous, milk, and vegetable diet, as opposed to a diet rich in albuminous materials.

The desiderata in the lactic bacillus employed are high vitality and a good degree of lactic acid producing power. These are best seen in the Caucasian or Bulgarian bacillus, isolated originally from Bulgarian maya, and described by Metchnikoff, Massol, and Boucard. When ingested by man, this bacillus is not destroyed in the intestine, but reaches the end of the colon in a living state, and is found in the stools. Though not normally an inhabitant of the human intestine, it readily becomes implanted there, and then acts efficiently against anaerobic bacteria. This organism may be used in conjunction with the organisms of Hüppe and Günther, and these are usually added for the improvement in flavour they impart to the soured milk. Therapeutically, however, only the bacillus of Massol is an efficient anti-putrefactive agent. Metchnikoff condemns the use of kephir and other doubly fermented milks containing yeasts and cocci on the grounds of their alcohol content and their irritating effect in some forms of enteritis.

Soured milk for medicinal use must be prepared from milk that is free from preservative and that has been rendered practically sterile by boiling; milk so treated and subsequently inoculated with the bacterial culture, is incubated at a temperature of 100° to 105° F. until the desired degree of acidity is reached. Curdling takes place when about 1 per cent of lactic acid is present in the milk, but this degree of acidity will be nearly doubled if sufficient time be allowed to elapse, though the flavour of the soured milk will be detrimentally affected. The necessity for initial sterilization of the milk will be realized when it is considered that the incubating process would at first encourage the multiplication of any extraneous organism present, and the ultimate destruction of such resistant pathogenic germs as those of tubercle and typhoid fever could not otherwise be ensured. The cultures are most conveniently employed in tablet form, which retain their activity for a long period, and possess every advantage over liquid cultures, except that they require a rather longer incubation period to produce the full degree of acidity. Patients who are unable to tolerate milk even in this readily digestible form are treated, in the opinion of some physicians, with equal success, by the administration of the tablets themselves, suitable saccharine matters being given at

the same time. When taken regularly, the bacilli commence to appear in the stools in about three days, and become established in the intestine in about eight days. The course of treatment usually lasts about twelve weeks without intermission.

CHEESE.

Cheese consists essentially of the curd and fat of the milk of any animal removed from the milk which has been curdled either by natural souring of the milk or by the action of rennet. The mass of curd and fat, after compression, is allowed to undergo certain fermentative changes, due to the action of micro-organisms or enzymes. In this country cheese is made entirely from cow's milk, and certain additions, such as colouring matter and salt, are regarded as quite legitimate. On the continent the milk of other animals, such as sheep and goats, is used for the manufacture of cheese. Cream is also used as the source of the cheeses known as cream cheeses. The nature of the decomposition which takes place in the ripening of cheese is but little known, but there is no doubt that amongst the principal of these is the degradation of proteid matter to compounds of much lower molecular weight, and containing much nitrogen. In such "ripe" cheeses mould is very common, and many cheeses are never eaten until they are very mouldy. The principal moulds existing in cheese are *aspergillus glaucus*, and *sporodonema casei*. When decomposition has proceeded very far large living organisms, such as the *acarus domesticus*, the ordinary cheese mite, are to be found, and are much appreciated by many cheese eaters. The following are the principal types of cheese met with in commerce:—

(1) English cheese; such as Cheddar, Cheshire, Stilton, Wensleydale, all being made from full cream milk.

(2) American cheese; usually made on the type of English Cheddar cheese, and made from full cream milk.

(3) Dutch cheese; usually made from partially skimmed milk.

(4) Gruyère; a Swiss cheese, made from goats' milk.

(5) Soft French cheeses; made from milk with cream added, such as Brie and Neufchatel.

(6) Ewe's milk cheese, of which Roquefort is a type.

No standard can at present be laid down for cheese, other than that it shall be the product of milk solely, or at all events with the small allowable additions of salt and colouring matter. The important point is that it shall be free from foreign fat, otherwise it must be sold as margarine cheese. Section 25 of the Sale of Food and Drugs Act, 1899, provides that—

"The expression 'margarine cheese' means any substance, whether compounded or otherwise, which is prepared in imitation of cheese, and which contains fat not derived from milk."

"The expression 'cheese' means the substance usually known as cheese containing no fat derived otherwise than from milk."

The following analyses of cheese are due to Woll ("Dairy Calendar," 223):—

	Water.	Casein.	Fat.	Sugar.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent
Cheddar	34.38	26.38	32.71	2.95	3.58
Cheshire	32.59	32.51	26.06	4.53	4.31
Stilton	30.35	28.85	35.39	1.59	3.83
Brie	50.35	17.18	25.12	1.94	5.41
Neufchatel	44.47	14.60	33.70	4.24	2.99
Roquefort	31.20	27.63	33.16	2.00	6.01
Edam	36.28	24.06	30.26	4.60	4.90
Swiss	35.80	24.44	37.40	—	2.36
Cream	38.60	25.35	30.25	2.03	4.07

Muter ("Analyst," x. 3) has published the following series of fuller analyses than the above:—

	Water.	Fat.	Lactic Acid.	Lactose.	Ash.		NaCl.	Fat.		Sap. Value.
					Insol.	Sol.		Insol. Acids.	Sol. Acids.	
American Cheddar	29.7	30.7	0.9	trace	2.16	1.54	1.2	89.98	3.3	220
Bondon (cream)	55.2	20.8	0.9	0.74	0.52	6.96	3.16	87.34	5.95	228
Camenbert	48.78	21.35	0.36	trace	0.16	8.64	3.46	87.15	6.09	229
Cheddar	33.40	26.6	1.53	—	2.3	2.0	1.52	87.66	5.60	227
Gloucester	37.2	22.8	1.8	—	2.56	2.0	1.64	87.00	6.28	229
Dutch	42.7	16.3	1.35	—	2.26	9.1	4.02	87.2	6.09	229
Gruyère	33.2	27.3	1.35	—	3.12	1.58	1.05	87.3	5.98	228
Roquefort	21.56	35.96	0.72	—	1.70	8.54	3.42	87.0	6.27	229
Stilton	28.60	30.70	1.08	—	1.80	2.22	0.75	86.2	7.02	231

A number of useful analyses are also published by Chattaway, Peannain and Moore ("Analyst," xix. 145).

	Water.	Fat.	Ash.	N.	Proteids.	Reichert-Meissl Value.	Valenta Test of Fat.
Cheddar	33.8	30.5	4.1	4.2	26.7	24.4	31° C.
Cheddar (Canadian)	33.3	30.6	3.6	4.34	27.6	24.0	41.5°
American	29.8	33.9	3.7	4.76	30.3	26.2	47.5°
Gorgonzola	40.3	26.1	5.3	4.36	27.7	22.1	26.5°
Dutch	41.8	10.6	6.3	5.11	32.5	27.0	40°
Gruyère	35.7	31.8	3.7	4.49	28.7	31.1	41°
Stilton	21.2	45.8	2.9	4.14	26.3	32	45.5°
Cheshire	37.8	31.3	4.2	4.03	25.7	31.6	43°
Gloucester	37.4	28.1	4.6	4.45	28.3	32.3	41°
Camenbert	43.4	22.6	3.8	3.83	24.4	35	33°
Parmesan	32.5	17.1	6.2	6.86	43.6	28	28°
Roquefort	29.6	30.3	6.7	4.45	28.3	36.8	19°
Double cream . . .	57.6	39.3	3.4	3.14	19.0	31.2	40°

The Adulteration of Cheese.—Cheese is adulterated by the addition of foreign fats, often in total substitution of the milk fat. If by cheese one understands a cheese made from full cream milk, the cheese made from skim milk would be regarded as adulterated, but until the Board of Agriculture, make regulations, as they are empowered to do by the Food and Drugs Act, as to standards for cheese, it appears to be legal to sell skim milk cheese as "cheese".

The use of foreign fats—such as lard and compositions of the margarine type, in the manufacture of "cheese"—forms the basis of a very large industry, especially in America, where this margarine-cheese is generally known as "filled" cheese.

The adulteration of cheese (gorgonzola) by the use of abnormally thick artificial rinds, composed of tallow, iron oxide and barium sulphate has recently formed the subject of successful prosecutions (see Vol. II, p. 29).

THE ANALYSIS OF CHEESE.

Moisture.—Two to three grms. are heated in a water oven for several hours until of constant weight. The loss is reckoned as water.

Mineral Matter.—The residue from the moisture determination is ignited at a low red heat and weight. To determine the salt, a separate portion should be charred at a low heat and the salt extracted by repeated boiling with distilled water and titrated with standard silver nitrate solution.

Fat.—Five grms. should be dried and rubbed down in a mortar with 20 grms. of ignited sand till a powdery mixture is obtained. This is then extracted in a Soxhlet with petroleum ether in the usual manner.

This solvent is preferable to ether, as ether dissolves appreciable quantities of lactic acid. Allen prefers to boil the powdered cheese with several portions of the solvent and decant each time. He finds that four boilings are sufficient to exhaust the cheese.

The fat may also be determined by the Werner-Schmidt method (see p. 50). Three grms. of the cheese should be boiled with 5 c.c. of water and 10 c.c. of concentrated HCl, till, with constant shaking, all but the fat is dissolved.

The Lythgoe-Babcock method is as follows: Take 5 to 6 grms. of the cheese in a tared beaker, add 10 c.c. of boiling water and stir with a rod until the cheese softens and an even emulsion is formed, adding a few drops of ammonia to aid the process. The beaker may be kept in hot water until the emulsion is complete and free from lumps. Then add about half of the 17.6 c.c. of the sulphuric acid regularly employed in the Babcock milk test (see p. 51), stir well, and pour into the Babcock bottle. Wash the beaker out with the remainder of the acid. Then proceed in the usual manner as in the centrifugal milk test, reading the amount of fat in the neck as usual.

Lactic Acid.—Ten grms. of the cheese are shredded and made up with water to 105 c.c. The mixture is heated to 50° and well shaken

for some time. The liquid is cooled, and filtered. Twenty-five c.c. of the filtrate is practically equivalent to 2.5 grms. of cheese. This quantity is titrated with decinormal alkali, using phenol-phthalein as indicator. Each c.c. of alkali required may be regarded as being equivalent to 0.009 grms. of lactic acid.

Milk Sugar.—Twenty-five grms. of cheese are divided as finely as possible and extracted by boiling with three successive quantities of 100 c.c. of distilled water. The mixed filtrates are, when cold, diluted to 250 c.c. and the milk sugar is determined in the ordinary way by titration against Fehling's solution.

Examination of the Fat.—All that is stated under butter fat applies to the examination of the fat extracted from the cheese, and a judgment as to the presence of foreign fatty matter must be based on the examination of the fat, especially by the Reichert process and the refractometer.

Detection of Skimmed Milk Cheese.—In a whole milk cheese, fat is almost invariably in excess of the proteids; when this is not the case, it is only slightly below the nitrogenous constituents. If the fat is materially below the proteids, the cheese has certainly been made from skim milk. In such cheeses, the fat will often fall as low as 5 to 15 per cent.

Determination of Nitrogenous Matter.—The total nitrogen may be determined in 2 grms. by Kjeldahl's process, and this, multiplied by 6.33, may be taken as the total "proteid matter".

Where a full examination of the nitrogenous matter is desired, the process of Van Slyke may be adopted. This is as follows:—

Place 25 grms. of the sample in a porcelain mortar and mix with the same amount of clear quartz sand. Transfer the mixture to a 450 c.c. Erlenmeyer flask and add about 100 c.c. of water at 50° C., keeping the temperature at 50° to 55° C. for half an hour, and frequently shaking. Transfer the liquid through an absorbent-cotton filter to a 500 c.c. graduated flask. Heat, shake, and decant from the residue repeatedly portions of water of 100 c.c., until the filtrate or water extract amounts to just 500 c.c. at room temperature, without taking into consideration the fat floating on the top; use aliquot parts of this water extract for the various determinations.

Water-soluble Nitrogen.—To determine the nitrogen use Gunning's method on 50 c.c. of the foregoing water extract corresponding to 2.5 grms. of cheese.

Nitrogen as Paranuclein.—To 100 c.c. of the above water extract (corresponding to 5 grms. of cheese) add 5 c.c. of a 1 per cent solution of hydrochloric acid. Keep the temperature at 50° to 55° until a clear liquid floats on the surface, showing that separation is complete. Filter, wash the precipitate with water, and employ the Gunning method to determine the nitrogen.

Nitrogen as Coagulable Protein.—Take the filtrate of the preceding determination and neutralize with dilute potassium hydroxide. Heat to the temperature of boiling water until any coagulum that there may be present completely settles. Filter, wash the precipitate and determine the nitrogen contained.

Nitrogen as Caseoses.—To the preceding filtrate add 1 c.c. of 50 per cent sulphuric acid saturated with zinc sulphate and warm to a temperature of about 70° C. till the caseoses settle out completely. Allow to cool, filter, and wash with a saturated solution of zinc sulphate made acid with sulphuric acid. Determine the nitrogen in the precipitate.

Nitrogen as Amides and Peptones.—Into a 250 c.c. graduated flask pass 100 c.c. of the aqueous extract of cheese. Add 1 grm. of sodium chloride and a solution containing 12 per cent of tannin until the clear liquid floating on the surface does not precipitate further. Dilute to the 250 c.c. mark, shake, pour upon a dry filter, and determine the nitrogen in 50 c.c. of the filtrate, which indicates the amount of nitrogen in the amido-acid and ammonia compounds. If the amount of nitrogen as ammonia separately determined, is deducted the difference is the amido-nitrogen.

Nitrogen as peptones can be obtained by deducting the total sum of the amounts of nitrogen, as paranuclein, coagulable proteins, caseoses, amido-bodies and ammonia from the whole amount of nitrogen in the aqueous extract.

Nitrogen as Ammonia.—Take 100 c.c. of the filtrate from the foregoing tannin-salt precipitation and distil into standardized acid, then titrate in the usual way.

Nitrogen as Paracasein Lactate.—To the residue, which is found insoluble in water when obtaining the aqueous extract, add several portions of a 5 per cent solution of sodium chloride. This forms a 500 c.c. salt extract of the same, in a similar way to that employed in preparing the water extract.

Take an aliquot part of this salt extract to determine the nitrogen.

Van Ketel and Antusch ("Nederl. Tydschr. Pharm." 1897, 82) affirm from the analysis of a number of cheeses that only about 80 per cent of the nitrogen is present in the form of proteids, the remaining 20 per cent existing as ammonia and amido-bodies. To determine the nitrogen present as ammonia, they distil the sample, which should have previously been powdered with the addition of sand, with water containing barium carbonate in suspension. Transfer the distillate into a measured quantity of standard sulphuric acid, boil, then neutralize the excess of acid with standard soda, using rosolic acid as an indicator. To determine the nitrogen present as amido-compounds, steep the powdered cheese with water for fifteen hours at ordinary temperatures. Add a little dilute sulphuric acid (1 : 4), then precipitate the peptones and proteids by phospho-tungstic acid. Filter off the precipitate, and wash with water containing a little sulphuric acid. Make up the filtrate to a definite amount, and determine the nitrogen in an aliquot part of the liquid by Kjeldahl's process, making allowance for the nitrogen existing as ammonia. To determine the peptones and albumoses together, boil the powdered cheese (mixed with sand as already described) with water; filter, leaving the undissolved casein and albumin. Add dilute sulphuric acid and phospho-tungstic acid to precipitate the peptones and albumoses in an aliquot part of the filtrate. Wash with acidulated water, then treat the precipitate

by Kjeldahl's process. The total amount of nitrogen in the cheese can be estimated by Kjeldahl's process and, after allowing for the nitrogen present in other forms, the balance is calculated to casein. A very small amount of indigestible casein is present.

Another and more elaborate method of distinguishing the various classes of nitrogenized compounds to be found in matured cheese is described by A. Stützer ("Zeit. Anal. Chem." 1896, xxxv. 493; "Analyst," xxii. 14). The following table of figures shows the results obtained by Stützer in three cases:—

	Camenbert.	Swiss.	Gervais.
	Per cent.	Per cent.	Per cent.
Water	50.90	33.01	44.84
Fat	27.30	30.28	36.73
Fat-free organic matter	18.66	31.41	15.48
Ash	3.14	5.30	2.95
The ash contained—			
Calcium	0.03	1.56	0.14
Phosphoric acid	0.76	0.82	0.23
Sodium chloride	2.21	1.56	0.76
Total nitrogen	2.900	5.072	1.923
Nitrogen as ammonia	0.386	0.188	0.031
" " amides	1.117	0.459	0.099
" " albumoses and peptones	0.885	0.435	0.298
" " indigestible matter	0.115	0.119	0.166
" " casein and albumin	0.397	3.871	1.139
In 100 parts of nitrogen there existed—			
As ammonia	13.0	3.7	1.6
" amides	38.5	9.0	5.2
" albumoses and peptones	30.5	8.6	15.5
" indigestible matter	4.0	2.4	8.6
" casein and albumin	14.0	76.3	69.1
Percentage of casein and albumin dissolved in pepsin solution—			
In 30 minutes	100	68	52
" 60 "	100	91	75

BUTTER.

Butter is the product obtained by churning milk, so that the fat globules adhere in a compact mass, together with a certain amount of water and non-fatty solids, the greater portion of the milk serum being removed by washing and mechanical means. More or less common salt is added, according to taste, the product being sold as salt butter or fresh butter according to the amount of salt it contains. Butter is one of the few articles of food for which a legal standard exists. The Board of Agriculture, acting under the powers conferred on them by section four of the Food and Drugs Act of 1899, framed regulations for the sale of butter in 1902. If any butter be sold containing more

than 16 per cent of water it shall be presumed to contain added water and therefore not to be genuine butter, until the contrary be proved. This point is dealt with fully in Vol. II. The average composition of normal butter, made from cow's milk is as follows :—

	Per cent
Water	12.00
Butter fat	86.80
Casein	0.50
Lactose	0.45
Mineral matter	0.25

Naturally, abnormal samples are to be met with, but these are usually due to the use of methods of manufacture not generally employed.

The determination of the proportions of the proximate constituents of butter presents no difficulties. The whole problem of butter analysis lies in the examination of the fat.

The Composition of Butter Fat.—Pure butter fat consists almost entirely of triglycerides of the fatty acids. Traces of cholesterol and colouring matter are also present, but the amount is rarely more than 0.5 per cent. In addition to the glycerides of oleic, palmitic and stearic acid, there are small quantities of the glycerides of arachidic, myristic and lauric acid. But the characteristic feature of butter fat is the comparatively large amount of glycerides of volatile fatty acids, amongst which are those of butyric, caproic, caprylic and capric acids (and traces of acetic acid). It is the decomposition of these latter, with the liberation of the volatile fatty acids that causes "rancidity" in butter.

According to Violette ("Journ. Soc. Chem. Ind." 1890, 1157) the following represent the percentage composition of butter fat :—

	1.	2.	3.	4.	5.	6.	7.	8.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Butyrim	6.94	6.09	6.28	5.76	5.28	5.49	5.45	5.00
Caproin	4.06	3.58	3.70	3.39	3.09	3.23	3.10	2.94
Glycerides of volatile solid acids	3.06	3.22	2.96	3.16	3.06	2.53	3.16	3.15
" of non-volatile acids	85.98	86.62	86.60	86.93	88.10	88.10	87.60	88.42

Other observers give the following values :—

	Bell.	Blyth.	Spallanzani.
	Per cent	Per cent	Per cent
Butyrim	7.01	7.7	5.08
Caproin			1.02
Caprylin and Caprin	2.28	0.1	0.31
Olein	37.73	42.2	
Palmitic, stearin, etc.	52.98	50.0	93.59

THE ANALYSIS OF BUTTER.

Determination of Water.—This is determined by heating 3 to 5 grms. in a flat capsule. A small glass rod should be weighed with the capsule, and the fat stirred repeatedly. The sample should be heated for from five to six hours. A fairly accurate and rapid method is to shake 10 grms. of butter with 30 c.c. of ether previously saturated with water. The separated aqueous liquid is run off into a graduated tube containing 5 c.c. of brine containing a drop or two of acetic acid. The increase of volume of the aqueous liquid represents nearly accurately the number of grms. of water in the 10 grms. of butter.

As mentioned above, butter should not contain more than 16 per cent of water. Where a higher percentage is present, it is nearly always the case that special methods have been used to incorporate a higher percentage of water with the fat. No properly made butter need contain more than 16 per cent. A practice started some few years ago of blending milk with butter. This, of course, resulted in getting considerably more than 16 per cent of water into the butter, and is practically tantamount to the addition of water. Legal decisions have now caused that it shall be sold under the qualified name of "milk-blended butter".

Martinez ("Land. Jahb." 1898, 773) reported on over 20,000 samples of *normally* made butter from various European countries, and finds no figures outside the limits 11.18 per cent and 13.99 per cent of water. Most Irish butter, which is made at higher temperatures than normal, contains up to 23 or 24 per cent of water, but it is necessary to disclose this fact when selling it.

Determination of Non-fatty Solids.—The portions of the sample used for the determination of water may be used for this determination. The dried residue should be repeatedly exhausted with warm petroleum ether, the liquid poured off each time, and when no further fat is extracted the non-fatty solids, consisting of lactose, casein, and mineral matter, are dried and weighed. If it be necessary to determine the casein separately, it may be done by washing the fat-free residue several times with water acidulated with acetic acid. Nearly pure casein is left behind. Accurate results are obtained by a determination of the nitrogen by the Kjeldahl process, and multiplying by 6.37.

Determination of Mineral Matter.—This, owing to its small amount, should be determined on the ether-insoluble residue of 10 grms. of the butter.

Great care must be taken not to ignite at too high a temperature lest sodium chloride should volatilize.

To determine the common salt present, the half-charred ash should be repeatedly extracted with hot distilled water and the sodium chloride determined either by titration or gravimetrically as silver chloride. Greater accuracy is attained by melting 10 grms. of the butter with 10 grms. of paraffin wax and well shaking the mixture with 50 c.c. of hot water acidulated with 1 c.c. of citric acid. The cake is well washed on cooling and the sodium chloride determined in

the liquid. No standard can be laid down for the amount of salt. It is a matter of taste. Bell found in 113 samples amounts varying from 0.4 to 9.20 per cent. If the non-fatty solids are, apart from the salt, not more than 1 to 1.5 per cent no further examination of these solids is necessary. An excess of non-fatty solids will at once call for special examination when starchy matters, etc., may be looked for.

Determination of the Fat.—Generally the difference figure will give the amount of fat with sufficient accuracy; but if a direct determination be required the ether extract is evaporated and the residue weighed. The examination of the butter-fat is described later (p. 95).

Added Colouring Matter.—Martin ("Analyst," xii. p. 70) gives the following details for the detection of added colouring matter to butter. He uses a mixture of 2 volumes of CS_2 and 15 volumes of alcohol. Twenty-five c.c. of this are shaken with 5 grms. of the butter, and after separation into the layers, the lower one will consist of the carbon bisulphide with the fat in solution, and the upper one will consist of alcohol containing artificial colouring matter if present. The following colours will react as indicated :—

Saffron.—If saffron be present, the alcoholic liquid will be coloured green by nitric acid and red by hydrochloric acid and sugar.

Turmeric.—Ammonia will turn the alcohol brown. Turmeric will also be detected by evaporating the alcohol, and boiling the residue with a few c.c. of dilute boric acid solution, and soaking a strip of filter paper in the liquid. On drying this will assume the usual red coloration, turning olive green on treatment with potash.

Coal-tar Dyes.—These may be detected by boiling wool fibres in the alcoholic extract diluted with water and acidulated with a few drops of HCl .

The following special tests should be used for certain colouring matters :—

Carrotin.—As this is more soluble in CS_2 than in alcohol, the following process should be used. Fifty grms. of the sample are melted, and 5 to 10 grms. of powdered fuller's earth stirred in. After well stirring the earth is allowed to settle and the warm fat poured off, 20 c.c. of benzol added, and after stirring, the liquid is decanted through a filter. This process is repeated until all the fat is removed and the precipitate washed on the filter with benzene. If the benzene be evaporated, and the residue shaken with alcohol containing a drop of dilute ferric chloride solution, and CS_2 , the alcohol will be coloured yellow, if carrotin be present.

Annatto.—Two to three grms. of the fat, freed from water and non-fatty solids, are warmed with a 2 per cent solution of sodium hydroxide. After well stirring, pour the mixture on to a wet filter in a hot funnel. In the presence of annatto the filter paper will absorb much of the colour, and become dyed a straw colour. The filtrate should be returned several times, if necessary, to the funnel, in order to thoroughly extract the melted fat. If the paper, after drying, turns pink when treated with a drop of stannous chloride solution, annatto is certainly present.

A confirmatory test for annatto is to soak a filter paper for twenty-

four hours in the solution rendered alkaline with a little Na_2CO_3 . The paper is stained brown in the presence of annatto, the colour changing to pink by the action of HCl .

Cornelison recommends the following process for the detection of artificial colouring matter ("Journ. Amer. Chem. Soc." 1908, 1478). Ten grms. of the dry filtered fat are shaken well in a separator with 10 to 20 c.c. of glacial acetic acid. At about 35° , the fat will separate almost completely, and the clear acid is drawn off. Natural butter gives a colourless liquid, which is unaltered by the addition of nitric or sulphuric acid. The acid extracts of butters containing annatto, turmeric or carotin are yellow in colour, changing to pink—especially with turmeric—by the addition of sulphuric acid. If methyl-orange be present it will respond to this reaction.

Leeds ("Analyst," XII. 150) dissolves 100 grms. of butter in 300 c.c. of petroleum ether (specific gravity = 0.638) in a separator, drains off the curd and water, and washes several times with water. The fat solution is kept at 0° for twelve to fifteen hours, so that the greater part of the solid glycerides crystallize out. The liquid is poured off and shaken with 50 c.c. of $\frac{N}{10}$ alkali to remove the colouring matters.

The aqueous layer is drawn off and exactly neutralized by $\frac{N}{10}$ hydrochloric acid, until a drop is just acid to litmus. The colouring matters are precipitated, contaminated with a trace of fat. The precipitate is dissolved in alcohol and a few drops tested with the reagents when the reactions given in the table on opposite page will be observed.

The usual preservative, when any is added, used for butter is boric acid or borax. A Departmental Committee on Food Preservatives in 1901 recommended that the only preservatives permitted to be used in butter or margarine should be boric acid or mixtures of boric acid and borax, and that no more than 0.5 per cent expressed as boric acid should be used. In general the methods described under milk may be adapted to the detection of preservatives in butter. In practice, however, boric acid, and, according to Hehner, sodium fluoride, are the only preservatives commonly to be found. For the determination of boric acid, 25 grms. of butter are mixed with 25 c.c. of a solution containing 6 grms. of lactose and 4 c.c. of normal sulphuric acid per 100 c.c. The mixture is placed in a water oven till the fat is just melted, and is then well stirred. The aqueous liquid is allowed to settle, and 20 c.c. are drawn off, a few drops of phenol-phthalein added, and the liquid is titrated with $\frac{N}{2}$ sodium hydroxide till a faint pink colour appears: add 12 c.c. of glycerine and again titrate till a pink colour appears. The difference in c.c. between the two titrations, less the amount of alkali required (as shown by a blank experiment) by the 12 c.c. of glycerine, is multiplied by 0.031. This gives the amount of boric acid in 20 c.c. of the liquid. So that this value multiplied by $\frac{100 + \text{per cent of water in the butter}}{20}$ will give the actual percentage present.

REACTIONS OF COLOURING MATTERS.

Colouring Matters.	Concentrated H_2SO_4 .	Concentrated HNO_3 .	$\text{H}_2\text{SO}_4 + \text{HNO}_3$.	Concentrated HCl .
Annatto	Indigo blue, changing to violet	Blue becoming colourless on standing	Same	No change, or only slight dirty yellow and brown
Annatto + decolorized butter	Blue, becoming green, and slowly changing to violet	Blue, then green and bleached	Decolorized	No change, or only slight dirty yellow
Turmeric	Pure violet	Violet	Violet	Violet, changing to original colour on evaporation of HCl
Turmeric + decolorized butter	Violet to purple	Violet to reddish-violet	Same	Very fine violet
Saffron	Violet to cobalt blue, changing to reddish-brown	Light blue changing to light reddish-brown	Same	Yellow, changing to dirty yellow
Saffron + decolorized butter	Dark blue changing quickly to reddish-brown	Blue, through green to brown	Blue, quickly changing to purple	Yellow becoming dirty yellow
Carrot	Umber brown	Decolorized	Do. with NO_2 fumes and odour of burnt sugar	No change
Carrot + decolorized butter	Reddish-brown to purple similar to turmeric	Yellow, and decolorized	Same	Slightly brown
Marigold	Dark olive green, permanent	Blue, changing instantly to dirty yellow green	Green	Green to yellowish-green
Safflower	Light brown	Partially decolorized	Decolorized	No change
Sudan orange	Pink	Pink	Pink	Pink
Martius yellow	Pale yellow	Yellow, reddish precipitate. Magenta at margin	Yellow	Yellow, precipitate treated with NH_3 and ignited; deflagrates
Victoria yellow	Partially decolorized	Same	Same	Same. colour returns on neutralizing with NH_3

Richmond and Harrison ("Analyst," xxvii. 179) recommend using 25 grms. of butter and enough water to make, with the water already present in the butter, 25 c.c. Ten to 15 c.c. of chloroform are then

added and the contents of the cylinder warmed, well shaken and allowed to separate. A measured portion is drawn off (each c.c. contains the boric acid of 1 gm. of the butter), made alkaline, evaporated, ignited, and the ash thoroughly extracted with hot water. The solution is rendered neutral to methyl orange, boiled to expel CO_2 and then titrated with standard $\left(\frac{\text{N}}{5}\right)$ alkali, after the addition of glycerol, with phenol-phthalein as indicator. One c.c. of $\frac{\text{N}}{5} \text{NaOH} = 0.0124$ gm. of boric acid.

Fluorides are detected, as recommended by Hehner, by separating the aqueous liquid from 50 grms. of butter, adding a little calcium chloride, boiling the liquid, and adding excess of Na_2CO_3 to precipitate calcium compounds. The precipitate is collected, washed, ignited and treated with hot dilute acetic acid. The insoluble residue is collected, ignited, and treated with strong H_2SO_4 in a platinum crucible, covered with a waxed glass on which a mark has been scratched. The crucible is stood on a sand bath for two hours, and in the pressure of fluoride, the glass will be distinctly etched.

It will be convenient to briefly discuss margarine, before passing on to the examination of butter-fat.

Various names have been used for butter substitutes, but to-day they are all covered by the word margarine, which is defined by section 3 of the Margarine Act, 1887 (50 and 51 Vict. c. 29) as follows:—

“The word margarine shall mean all substances, whether compounds or otherwise, prepared in imitation of butter, and whether mixed with butter or not, and no such substance shall be lawfully sold, except under the name of margarine, and under the conditions set forth in this Act.” By section 8 of the Sale of Food and Drugs Act, 1889, no margarine may be sold the fat of which contains *more* than 10 per cent of butter fat. This wholesome restriction is in the interests of the poorer classes, who may know that, whatever assurances the vendor might give, they can never get legitimately more than 10 per cent of butter fat in margarine.

The manufacture of margarine is to-day an enormous industry, and as the fats usually employed are now of a perfectly wholesome nature, there is no doubt that the industry has benefitted the poorer classes to a considerable extent. In this country a very large proportion of the margarine consumed is manufactured from the fat of cocoanuts. In the United States animal fats are more largely employed, together with such oils as cotton seed, arachis or sesame. It is not necessary to discuss the manufacture of margarine: the fat after suitable treatment is churned up with water, a little colouring matter, sometimes a little milk or butter, salt, etc., etc., until the proper consistency and the desired flavour are obtained. The actual composition of margarine is, of course, very varied. Many samples are made up, merely with regard to the flavour and appearance of the finished product. But the fact that cocoanut fat contains a large proportion of the glycerides of volatile fatty acids has caused a certain class of

manufacturer to adjust his formulæ so that the composition of his margarine may be such as to yield analytical results similar to those of butter. It cannot be denied that the use of cocoanut fat has caused a large increase in the adulteration of commercial butter.

The analyst will have frequently to decide (1) whether butter contains any margarine, (2) whether margarine contains more than 10 per cent of butter fat.

In regard to the analysis of margarine, there is nothing to be added to the details given for pure butter, so far. It is only in regard to the characters of the fat, that the analyst will be able to base an opinion as to the presence or absence of margarine in samples of butter.

THE ANALYSIS OF BUTTER FAT.

The fat should be separated from the water and non-fatty solids by allowing the butter to remain melted for a short time, when the fat is poured off through a dry filter in a hot water funnel. The following determinations are then necessary.

Specific Gravity.—It is usual to determine this value at 100° F. (37·8° C.), taking the specific gravity of water at that temperature as unity. At this temperature the specific gravity of genuine butter, $D \frac{37\cdot8^{\circ} \text{C.}}{37\cdot8^{\circ}}$ varies between the limits 0·910 to 0·9135. Some observers prefer to take the specific gravity at 100° C., and adopt water at 15·5° C. as unity. The specific gravity, $D \frac{100^{\circ} \text{C.}}{15\cdot5^{\circ}}$ varies from 0·8668 to 0·8705. Most adulterants lower the specific gravity, but cocoanut oil—which is the basis of much of the commercial margarine of to-day—has a rather higher specific gravity (up to 0·9175) at 37·8° C. Judicious mixtures, however, can be prepared with the same $\frac{37\cdot8^{\circ}}{37\cdot8^{\circ}}$ specific gravity as butter fat, so that this, as all other tests for butter, will only give an indication to be judged in combination with other results.

Melting-point.—The melting-point of butter fat, determined in very thin tubes, varies from 29° to 33°, rarely up to 34°. The insoluble fatty acids separated in the usual manner melt at 39° to 44° or rarely up to 45°.

Iodine Value.—The iodine value of butter fat (see p. 628) as determined by Hübl's solution usually varies from 28 to 34, but 26 to 28 are figures recorded by trustworthy observers. Cocoanut fat has a much lower iodine value, but such fats as cotton seed, with very high iodine values, can be used in small quantities to adjust this figure.

Saponification Value.—This figure varies, for pure butter fat, between the limits 220 to 234. Here again mixtures of other fats are easily made which have the same saponification value as that of pure butter fat.

Avi-Lallemant ("Zeit. Unter. Nahr. Genuss." 1907, **14**, 317) recommends the following method in the examination of the fatty acids of butter. Two grms. are saponified with alcoholic KOH, the liquid

neutralized with $\frac{N}{2}$ HCl, alcohol expelled, the soap dissolved in boiling water, and the boiling solution (150 to 180 c.c.) treated with 50 c.c. of a solution of 2.5 BaCl₂ per litre. The flask is left on the water bath for fifteen minutes, the contents cooled, made up to 250 c.c. and filtered. The barium remaining in solution is precipitated from 200 c.c. of the filtrate, which is acidified with HCl, by H₂SO₄ and weighed as BaSO₄. The amount of barium so found calculated as BaO is multiplied by 1.25, since only 200 c.c. of the filtrate were used, and this subtracted from the amount of BaO originally used, calculated to 1 gm. of fat, gives the amount of BaO which has combined with the fatty acids to give insoluble barium salts, i.e. = the "insoluble baryta value". If the saponification value of the fat be calculated into mgr. of BaO, this minus the insoluble baryta value—is the "soluble baryta value". The following values are obtained from pure butter and certain other fats:—

	Insoluble BaO Value.	Soluble BaO Value.
Butter	247.4 to 254.8	50.8 to 76.7
Sesame oil	251.9	3.3
Cotton-seed oil	256.9	6.6
Cocoonut oil	296.5 to 299.2	54.1 to 57.6
Lard	257.4	7.6
Beef tallow	264.1	6.2

Refractive Values.—The absolute refractive index of a given substance is a far more more scientific figure than any empirical values, such as the scale readings of instruments known as butyro-refractometers, etc. At the same time these instruments have come into use to a very large extent, and the values recorded by them are of considerable value, and being well established call for general recognition.

The following are the refractive indices of pure butter fat and certain other oils which may enter into the composition of butter substitutes, as determined by the author:—

Butter fat at 25° C.	. . .	1.4587 to 1.4615	(20 samples)
" " 38°	. . .	1.4540 " 1.4569	(20 ")
Cocoonut " 38°	. . .	1.4500 " 1.4515	(10 ")
Lard " 38°	. . .	1.4490 " 1.4505	(10 ")
Cotton seed " 38°	. . .	1.4660 " 1.4680	(10 ")
Suet " 38°	. . .	1.4605 " 1.4620	(5 ")

The butyro-refractometer is an instrument manufactured by Zeiss, similar to the ordinary refractometer, but graduated in arbitrary scale divisions. It has been said that the differences in the dispersive values of various fats is such that the critical line, seen in the refractometer of butter, is colourless, whilst that of other fats is blue. In the author's opinion this point is of no value at all, and only the quanti-

tative values are of importance. If a reading on this instrument be taken at one temperature, and it is desired to correct this from another temperature, 0.55° should be added for every 1° C. by which the temperature of observation is reduced or added for every degree by which it is increased. According to Wollny pure butter gives a scale reading of 49.5 to 54 degrees at 25° C. At 38° the readings vary from 42 to 46° . The value at 25° , however, rarely exceeds 52.5° , and at 38° , 44.5° . Coconut fat has a value 34 to 37° at 40° C. But, of course, mixtures can easily be made having the same refractive index as that of butter fat. A butter showing values outside the limits 49 to 54 at 25° may be condemned as adulterated. The table on pages 98, 99 gives the correspondence between the refractive index and the butyro-refractometer scale of reading. The figures given across the table are those in the first place of decimals corresponding to the figures in the fourth place of decimals of the refractive indices. An asterisk in these figures indicates that the figure has to be added to the scale reading of the next lower line.

Another instrument used in this respect is the oleo-refractometer, devised by Amagat and Jean. It is, like the above, an instrument graduated on a purely empirical scale. In this instrument a zero point exists, and readings to the right of this are designated + whilst those to the left are called -. Pure butter fats give a reading of -26° to -34° with an average of about -30° ; most vegetable fats give + readings, whilst coconut oil behaves like animal fats and gives a reading up to -58° .

The refractive index of the fatty acids (insoluble) of butter varies from 1.4370 to 1.4390 at 60° C., whereas that of the insoluble fatty acids of coconut oil never rises above 1.4301 at the same temperature.

The Volatile Fatty Acids.—The most valuable determination in connexion with the examination of butter fat is that of the volatile fatty acids, although even the value of this must not be over-estimated, on account of the fact that coconut fat contains so high an amount of volatile acids. Various modifications of the Reichert process exist, but as the following details of working have been agreed upon by official and unofficial analysts, they may be taken as practically official for the purposes of the Food and Drugs Acts. It is to be remembered that the Reichert-Wollny (or Reichert-Meissl) values refer to 5 grms. of the fat, whilst the Reichert values refer to 2.5 grms. only. To connect the lower with the higher value a slight correction is necessary, and it is usual to multiply it by 2.2. The following process has taken into account the errors in the original process as formulated by Wollny ("Journ. Soc. Chem. Ind." 1887, 831).

The details of this semi-official process are as follows:—

Five grms. of the dry fat are placed in a 300 c.c. flask (see Fig. 100) and 2 c.c. of an aqueous solution of NaOH (1 gm. in 1 c.c.), free from carbonate, are added, with 10 c.c. of 92 per cent alcohol (by volume). The mixture is heated under a reflux condenser on a water bath for fifteen minutes. The alcohol is then removed by heating on

TABLE OF REFRACTIVE INDICES AND REFRACTOMETER NUMBERS.

Ref. Index.	4th Decimal of Refractive Index.												Ref. Index.
	Scale No.	0	1	2	3	4	5	6	7	8	9	Scale No.	
1.422	0	0	1	2	4	5	6	7	9	*0	*1	0	1.422
1.423	1	2	4	5	6	7	9	*0	*1	*2	*4	1	1.423
1.424	2	5	6	7	8	*0	*1	*2	*3	*5	*6	2	1.424
1.425	3	7	8	*0	*1	*2	*3	*5	*6	*7	*8	3	1.425
1.426	5	0	1	2	4	5	6	7	9	*0	*1	5	1.426
1.427	6	2	4	5	6	8	9	*0	*1	*2	*4	6	1.427
1.428	7	5	6	7	9	*0	*1	*2	*4	*5	*6	7	1.428
1.429	8	7	9	*0	*1	*2	*4	*5	*6	*8	*9	8	1.429
1.430	10	0	1	3	4	5	6	7	9	*0	*1	10	1.430
1.431	11	3	4	5	6	8	9	*0	*2	*3	*4	11	1.431
1.432	12	5	7	8	9	*1	*2	*3	*5	*6	*7	12	1.432
1.433	13	8	*0	*1	*2	*4	*5	*6	*7	*9	*0	13	1.433
1.434	15	1	3	4	5	6	8	9	*0	*2	*3	15	1.434
1.435	16	4	6	7	8	*0	*1	*2	*4	*5	*6	16	1.435
1.436	17	8	9	*0	*2	*3	*4	*5	*7	*8	*9	17	1.436
1.437	19	1	2	3	5	6	7	8	*0	*1	*3	19	1.437
1.438	20	4	5	6	8	9	*1	*2	*3	*4	*6	20	1.438
1.439	21	7	8	*0	*1	*2	*4	*5	*6	*7	*9	21	1.439
1.440	23	0	2	3	4	5	7	8	9	*1	*2	23	1.440
1.441	24	3	5	6	7	8	*0	*1	*2	*4	*5	24	1.441
1.442	25	6	8	9	*1	*2	*3	*5	*6	*7	*9	25	1.442
1.443	27	0	1	3	4	5	7	8	9	*1	*2	27	1.443
1.444	28	3	5	6	7	9	*0	*2	*3	*4	*6	28	1.444
1.445	29	7	9	*0	*1	*3	*4	*6	*7	*8	*9	29	1.445
1.446	31	1	2	4	5	6	8	9	1	*2	*3	31	1.446
1.447	32	5	6	8	9	*0	*2	*3	*5	*6	*7	32	1.447
1.448	33	9	*0	*2	*3	*4	*6	*7	9	*0	*1	33	1.448
1.449	35	3	4	6	7	8	*0	*1	3	*4	*5	35	1.449
1.450	36	7	8	*0	*1	*2	*4	*5	*7	*8	*9	36	1.450
1.451	38	1	2	3	5	6	7	9	*0	*2	*3	38	1.451
1.452	39	5	6	7	9	*0	*1	*3	*4	*6	*7	39	1.452
1.453	40	9	*0	*1	*3	*4	*5	*7	*8	*0	*1	40	1.453
1.454	42	3	4	5	7	8	*0	*1	*3	*4	*6	42	1.454
1.455	43	7	9	*0	*2	*3	*4	*6	*7	*9	*0	43	1.455
1.456	45	2	3	5	6	7	9	*0	*2	*3	*4	45	1.456
1.457	46	6	7	9	*0	*2	*3	*5	*6	*7	*9	46	1.457
1.458	48	0	2	3	5	6	8	9	*1	*2	*4	48	1.458
1.459	49	5	7	8	*0	*1	*2	*4	*5	*7	*8	49	1.459
1.460	51	0	1	3	4	6	7	9	*0	*2	*3	51	1.460

TABLE OF REFRACTIVE INDICES AND REFRACTIVE NUMBERS—
Continued.

Ref. Index.	4th Decimal of Refractive Index.												Ref. Index.
	Scale No.	0	1	2	3	4	5	6	7	8	9	Scale No.	
1.461	52	5	7	8	*0	*1	*3	*4	*6	*7	*9	52	1.461
1.462	54	0	2	3	5	6	8	*0	*1	*3	*4	54	1.462
1.463	55	6	7	9	*0	*2	*3	*5	*6	*8	*9	55	1.463
1.464	57	1	3	4	6	7	9	*0	*2	*3	*5	57	1.464
1.465	58	6	8	9	*1	*2	*4	*5	*7	*8	*0	58	1.465
1.466	60	2	3	5	6	8	9	*1	*2	*4	*5	60	1.466
1.467	61	7	8	*0	*2	*3	*5	*6	*8	*9	**1	61	1.467
1.468	63	2	4	5	7	8	*0	*2	*3	*5	*7	63	1.468
1.469	64	8	*0	*1	*3	*4	*6	*7	*9	**1	**2	64	1.469
1.470	66	4	5	7	8	*0	*2	*3	*5	*7	*8	66	1.470
1.471	68	0	1	3	4	6	7	9	*1	*2	*4	68	1.471
1.472	69	5	7	9	*0	*2	*3	*5	*7	*8	*0	69	1.472
1.473	71	1	3	4	6	8	9	*1	*2	*4	*5	71	1.473
1.474	72	7	9	*0	*2	*3	*5	*7	*8	**0	**1	72	1.474
1.475	74	3	5	6	8	*0	*1	*3	*5	*6	*8	74	1.475
1.476	76	0	1	3	5	7	8	*0	*2	*3	*5	76	1.476
1.477	77	7	9	*1	*2	*4	*6	*7	*9	**1	**2	77	1.477
1.478	79	4	6	8	*0	*1	*3	*5	*6	*8	**0	79	1.478
1.479	81	2	3	5	7	9	*0	*2	*4	*5	*7	81	1.479
1.480	82	9	*1	*2	*4	*6	*8	*9	**1	**3	**5	82	1.480
1.481	84	6	8	*0	*2	*3	*5	*7	*9	**0	**2	84	1.481
1.482	86	4	6	7	9	*1	*3	*5	*6	8	**0	86	1.482
1.483	88	2	3	5	7	9	*1	*2	*4	*6	*8	88	1.483
1.484	90	0	2	3	5	7	9	*1	*2	*4	*6	90	1.484
1.485	91	8	*0	*1	*3	*5	*7	*9	**0	**2	**4	91	1.485
1.486	93	6	8	*0	*1	*3	*5	*7	*9	**0	**2	93	1.486
1.487	95	4	6	8	*0	*1	*3	*5	*7	*9	**0	95	1.487
1.488	97	2	4	6	8	*0	*1	*3	*5	*7	9	97	1.488
1.489	99	1	2	4	6	8	*0	*2	*3	*5	7	99	1.489
1.490	100	9	*1	*3	*4	*6	*8	**0	**2	**4	**6	100	1.490
1.491	102	7	9	*1	*3	*5	*6	*8	**0	**2	**4	102	1.491
1.492	104	6	8	105	—	—	—	—	—	—	—		

the water bath, and then 100 c.c. of hot water (which has been well boiled for ten minutes to remove CO_2) are added and the flask warmed until the soap is dissolved. Forty c.c. of normal H_2SO_4 are then added and a few fragments of pumice stone, and the flask immediately connected with the condenser. The flask is supported on a circular piece of asbestos 12 cms. in diameter, having a hole in the centre 5 cms. in diameter, and is just heated so that the insoluble fatty

acids melt, and then heated until the liquid boils and 110 c.c. of liquid are collected, the distillation lasting about thirty minutes. The distillate is shaken and filtered, and 100 c.c. of the filtrate titrated with decinormal alkali, phenol-phthalein being used as indicator. A blank experiment should be conducted on the material employed, but the amount of decinormal alkali required should not exceed 0.3 c.c. This should be deducted from the result obtained, and the number of c.c. thus found, multiplied by 1.1, is the Reichert-Wollny number, which is a measure of the soluble volatile fatty acids.

The following is a diagram of the apparatus employed.

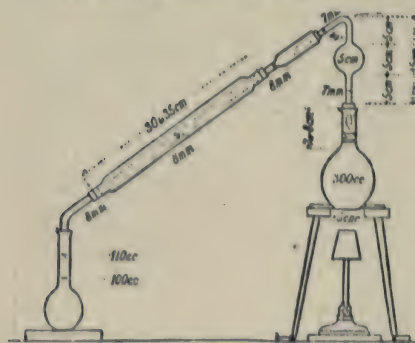


FIG. 8.—Reichert-Wollny apparatus.

It is admitted on all hands that this figure may vary within wide limits, and that unless a very low standard be adopted, *occasionally* a genuine butter may be condemned. The usual limit adopted as a minimum is from 23 to 25; in this country the usual figure is 24. Since genuine butters give figures up to 33 and higher, it is obvious that this limit errs on the side of giving the benefit of the doubt to many samples of butter. Coconut oil gives a Reichert-Wollny value up to 8, hence mixtures of genuine butter with values of 28 to 30 with a considerable amount of coconut fat will satisfy this accepted limit of 24.

The majority of the modifications of the Reichert process are not of much importance, but a few contributions to the question have recently been made which are of considerable interest.

Blichfeldt has recently proposed ("Journ. Soc. Chem. Ind." 29, 792) the following method, depending on the differences in the amount of soluble and insoluble silver salts of the volatile fatty acids in butter and coconut fats:—

By this method the fat is saponified by a mixture of aqueous potash and glycerol, and the fatty acids are liberated by acidification with sulphuric acid. The resulting mixture is distilled in a specially designed apparatus, in which the connecting tube, condenser tube, and receiver, are all in one place. The distillate is treated with an excess of decinormal soda solution and transferred to a 200 c.c. measuring flask. The total volatile acids are determined by titrating back with decinormal sulphuric acids. The neutral solution is now treated with excess of decinormal silver nitrate solution, and 10 per cent of solid sodium nitrate is dissolved in the liquid in order to salt out all the sparingly soluble silver salts. After making up to 200 c.c., the precipitated silver salts are filtered off, and the excess of silver nitrate is determined volumetrically in the filtrate in the following manner. A

slight, known excess of decinormal sodium chloride solution is added, and the excess titrated back with decinormal silver nitrate solution. This will allow the original excess of silver nitrate to be calculated. The volatile fatty acids thus determined as soluble and insoluble silver salts are shown in the following table:—

Substance.	Volatile Acids in terms of Decinormal NaOH.			Silver Precipitation in terms of Decinormal Solution.		
	Total.	Soluble.	Insoluble.	Total.	Soluble.	Insoluble.
Butter	32	28	4	32	29	3
Cocoanut oil	20	6	14	20	4	16
Palm kernel oil	15	5	10	15	3	12

The ratios of soluble to insoluble silver salts derived from butter and cocoanut fats respectively differ considerably from one another, and afford a ready means of determining the substances in presence of one another. The method, however, makes no distinction between cocoanut oil and palm kernel oil.

Kirschner ("Zeit. Nahr. Genuss." 1905, **9**, 67) proposes the following method which is itself a modification of one previously suggested by Gensen. The distillate obtained in the Reichert-Wollny process, is filtered as usual and 100 c.c. titrated with decinormal baryta. To the neutral solution, 0.5 gm. of silver sulphate is added, and the mixture shaken from time to time during one hour and then filtered. The filtrate measures just over 100 c.c. Exactly 100 c.c. of this are placed in a flask, 35 c.c. of water and 10 c.c. of dilute H_2SO_4 added, and the whole distilled until 110 c.c. have been collected. This is again filtered, 100 c.c. of the filtrate are titrated with decinormal baryta and the result calculated for 5 grms. of fat. It is claimed by the author that the elimination of the acids precipitable by silver accentuates the difference between pure butter and a mixture with cocoanut fat.

Monhaupt ("Chem. Zeit." 1909, **33**, 305) claims that small quantities of butter fat can thus be detected in cocoanut fat preparation. He gives the following figures for margarines containing from 15 to 25 per cent of cocoanut fat and 1 to 2 per cent of butter fat:—

Per cent Cocoanut Fat.	Per cent Butter Fat.	Reichert-Wollny No.	Kirschner Value.
15	0	0.50	0.24
	1	0.72	0.39
	2	0.99	0.62
25	0	0.88	0.30
	1	0.99	0.55
	2	1.21	0.69
35	0	1.10	0.36
	1	1.32	0.63
	2	1.50	0.81

He states that if 20 grms. of fat be used, more marked differences still will be obtained. For further details, the original paper should be consulted.

Paal and Amberger ("Zeit. Untersuch. Nahr. Genuss." 1909, 17, 1) have recommended the quantitative determination of the volatile fatty acids which are precipitable by cadmium sulphate. As cadmium butyrate and caproate are soluble in water, this method deals mainly with the insoluble volatile fatty acids.

They use 2.5 grms. only of the fat, and after distillation of the fatty acids from the aqueous mixture, a little alcohol is distilled through the condenser, in order to wash away any deposited fatty acids. After titration, the exactly neutralized fatty acids are precipitated by the addition of 2 to 4 c.c. of a 20 per cent solution of cadmium sulphate. The precipitate is collected, washed with 50 c.c. of water, dried at 102° for one hour and weighed. The weight in milligrams is called the cadmium value of the fat. In pure butter this varies between 70 to 90, or in rare cases, due to abnormal feeding, it may rise to 100 or just over. Coconut fat has a "cadmium value" of 440 to 470.

The processes of Blichfeldt, Kirschner, Paal and Amberger are based on the observations of Polenske, who showed that the greater part of the volatile acids of butter are soluble, whereas the reverse is the case with coconut oil. Polenske's observations showed that in thirty-one samples of butter fat, the Reichert-Meissl values of which varied from 23.3 to 30.1, the amount of decinormal alkali required for neutralization of the insoluble acids was only from 1.5 to 3.0, whereas in samples of coconut oil, the Reichert-Meissl values of which varied from 6.8 to 7.7, the insoluble volatile acids required from 16.8 to 17.8 c.c. of decinormal alkali.

Polenske thereupon termed the number of c.c. thus required to neutralize the insoluble volatile acids of 5 grms. of the fat, the "new butter value".

The details of the process adopted by him are as follows:—

Five grms. of the butter fat are saponified by the Leffman-Beam process, using 20 grms. of glycerine and 2 c.c. of 50 per cent NaOH solution, in a 300 c.c. flask over a naked flame. The solution is allowed to cool below 100°, 90 c.c. of water are added, and the mass dissolved by warming on a water bath to 50°. The solution must be nearly colourless, otherwise the experiment must be repeated. To the warm solution 50 c.c. of sulphuric acid solution (2.5 per cent) are added, and some powdered pumice stone. The flask is at once connected with the condenser (in order to obtain absolutely concordant results, the apparatus should be of the exact dimensions given in the original paper—"Arbeit. aus dem Kaiser. Gesundheitsamte," 1904, 545—but the apparatus illustrated on p. 100 is so near to it that it may be used in this process with confidence). The liquid is distilled until 110 c.c. are collected in nineteen to twenty minutes. The receiving flask is now removed and a 25 c.c. cylinder substituted. The receiving flask is now stood in water at 15°, and after fifteen minutes the consistency of the insoluble acids which are floating on the surface noted. Pure butter acids are solid, whereas in the presence of 10 per

cent of cocoanut oil, the acids no longer solidify. The contents of the flask are mixed by merely turning the flask upside down several times and then 100 c.c. are filtered off, and the Reichert-Meissl value determined by titration. The insoluble acids collected on the filter are three times washed with 15 c.c. of water, which have been passed successively through the tube of the condenser, the 25 c.c. measuring cylinder and receiving flask.

Finally three portions of 15 c.c. each of 90 per cent alcohol are passed through the condenser, the measuring cylinder and the receiving flask, and each portion passed through the filter, which is allowed to drain before the next portion is added. The alcoholic filtrate is then titrated with decinormal alkali.

In thirty-four samples of pure butter Polenske found 1.35 to 3.0 as the insoluble volatile fatty acid figure, whereas, as mentioned above, the value reaches to 17.8 for cocoanut oil.

Polenske claims to be able to calculate the approximate amount of cocoanut oil present in a mixture, on the basis of an experimental table, and assumes that each 1 per cent of added cocoanut fat increases the insoluble acid value by 0.1. But this is based on two fallacies, firstly that the amount of soluble fatty acids and insoluble fatty acids in genuine butter fat are in definite ratio, and secondly, that one is dealing with a mixture of butter fat and cocoanut oil only. Still the process is of distinct value, but the results require careful interpretation.

Shrewsbury and Knapp ("Analyst," xxxv. 385) have proposed a process for the determination of cocoanut fat, which certainly gives very promising results. The fatty acids of this fat contain much lauric and myristic acid, which are not present in most other fats, and which are practically insoluble in water, but soluble in dilute alcohol. Their process, which is similar to the less useful one of Vandam ("Analyst," xxvi. 320), is as follows: 5 grs. of the filtered fat are saponified with 20 c.c. of glycerol soda as in the Reichert process, and the soap diluted with boiling water and at once transferred to a separator, using 200 c.c. of water in all; 5 c.c. of H_2SO_4 (1 in 4 of water) are added and the mixture well shaken for 60 seconds. The liquid is allowed to stand for 5 minutes, and the water run off from the insoluble acids. These are dissolved in the separator in 50 c.c. of methylated spirit, the liquid run into a flask and heated, adding a fragment of pumice to prevent bumping, 36 c.c. of cold water are then poured into the separator, and the boiling alcoholic solution poured into it. The mixture is poured back into the flask to wash it, again transferred to the separator, shaken for 30 seconds, left for 3 minutes, and 70 c.c. of the clear solution run off and titrated with decinormal soda, using phenolphthalein as indicator.

The figures thus found for the acid value of the 70 c.c. so obtained vary from 23.6 to 31.2, with an average of 27.7 c.c. of $\frac{N}{10}$ alkali for pure butters. Cocoanut oil, on the other hand, gives a value of about 163 c.c. of $\frac{10}{N}$ alkali. By adopting the values of 32 for pure butter

and 16.3 for cocoanut oil, a fair estimation of the amount present is obtained.

Valenta's Test.—After melting the butter fat, filter at as low a temperature as possible, then dry still further by again filtering through a dried filter-paper. Weigh 2.75 grms. of the fat and place in a stoppered test tube, and add 3 c.c. of 99.5 per cent acetic acid. Place the tube in a beaker of water, gradually heating the water until the solution becomes clear on shaking the tube. Notice carefully the temperature. The following figures represent the temperatures for butter fat and margarine respectively:—

	Maximum.	Minimum.	Average.
Margarine	39°	29°	36°
Butter fat	97°	94°	95°

In order to avoid any mistake always test the acetic acid first on a sample or samples of genuine butter fat. Jean prefers to determine the amount of acetic acid dissolved. He weighs about 8 c.c. of the fat into a graduated test tube 1 cm. in diameter, which is placed in water at 50° C. He then removes the excess of fat by means of a pipette until the fat measures 3 c.c. at 50° C., and adds 3 c.c. of glacial acetic acid (specific gravity 1.0565) which has been measured at 22°. The contents are then warmed for a few minutes, and, after inserting a cork in the test tube, well shaken. The tube is then immersed in the water at 50° C., and the amount of undissolved acetic acid determined. Nine samples of butter averaged 63.33 per cent of acetic acid dissolved; margarines vary from 27 to 32 per cent. The turbidity test and the amount of acetic acid dissolved can obviously be done on the same sample at the same time.

M. Crismer suggests a turbidity test bearing the name of "the critical temperature of dissolution". This differs from the Valenta test, but is doubtless of the same value. He proceeds as follows; 0.5 c.c. of filtered fat is weighed into a tube of small diameter; 0.75 c.c. of alcohol, specific gravity 0.7967 (containing 0.9 per cent of water) is added. The tube is hermetically sealed and fastened to the bulb of a thermometer by means of a platinum wire. The bulb and wire are then placed in a small sulphuric acid bath, and the temperature is raised gradually until the meniscus separating the two layers becomes a horizontal plane. The thermometer and tube must now be taken out of the bath and the tube well shaken to mix the two liquids together. They are again immersed in the bath and shaken all the time whilst the temperature is allowed to fall. Immediately a marked turbidity is to be seen, the temperature is noted. Genuine butters gave from 51° to 57° C., margarines varied from 69° to 78°; with alcohol of specific gravity 0.8195 containing 8.85 per cent of water butters give 98° to 105.5° C. and margarines 109° to 124° C.

Lewkowitsch considers that if an absolute test for the presence of a vegetable fat in butter be required, when other tests fail to decide the

question, the phytosteryl acetate test may be successfully employed and gives decisive results (p. 630). A very small amount of paraffin wax will, however, interfere with this test.

The amount of insoluble fatty acids in pure butter fat, which is determined by saponification and separating the fatty acids in the usual manner, should not exceed 89.5 per cent and is rarely more than 88 per cent. Coconut oil contains a low percentage of insoluble fatty acids, but nearly all other oils contain considerably more than 90 per cent, cotton-seed oil containing as much as 95 to 96 per cent. A butter yielding over 90 per cent is undoubtedly adulterated.

In America numerous empirical methods of examining butter are practised, such as the foaming or failure to foam of the sample when treated over a flame; the ease with which the fat mixes with milk or not, etc., but these are not of any serious value, and need not be discussed as methods of analysis.

Microscopical Examination of Butter.—With an ordinary light, and a low power of from about 120 to 150 diameters much information can be gained from a sample. A small portion on the edge of a knife blade is placed on the glass slide and gently pressed into a thin film by means of the cover glass. The difference between genuine and renovated butter is easily seen. The fat film of fresh butter is much more transparent than that of renovated, and the curd of the genuine butter fat is much more finely divided throughout the mass and the field is much more even than that of the renovated, the latter often showing large and opaque patches of curd throughout the field.

When a renovated butter sample, i.e. a "process butter"—rancid butter, melted and made palatable by blowing steam through it, etc., mounted as above—is examined by reflected light, obtained by turning the microscope mirror in such a way as not to transmit light through the instrument, a very dark and scarcely perceptible field is to be seen, whilst the above-mentioned opaque patches of curd are distinctly seen as white masses against a dark background.

With Polarized Light.—The crystalline structure of fat once melted and afterwards cooled is easily seen by the microscope which, as has already been stated, is useful in determining whether or not a fat has been melted, especially when examined by polarized light. This fact has been made use of for a long time in the identification of butter and oleomargarine by the microscope.

Pure butter, not previously melted, should show no crystalline structure when seen by polarized light between crossed Nicols under a low magnification, and it should be uniformly bright throughout. If the selenite plate is used, there should be an evenly coloured field with fat crystals entirely absent. With process butter or oleomargarine, previously melted then cooled, the crystalline structure presents a marked appearance, which is more or less mottled when viewed by polarized light, and if the selenite plate is used there is quite a play of colours.

There are various circumstances which may affect the reliability of the polarized light test. These distinctive features, already described, are particularly obvious in cold weather. The appearance of

pure butter is quite blank, whilst oleomargarine has a much more mottled appearance than renovated butter. An expert cannot always detect these well-defined points of variation in practice. Sometimes pure butter will show a somewhat mottled field owing to a slight crystallization at some previous time. In the summer, for example, these distinctions between pure and adulterated butter as indicated by polarized light, are not as reliable when the butter easily melts at ordinary temperature, as they are in winter. It is necessary, therefore, that both the collector of samples and the analyst should keep the samples to be examined from melting under ordinary conditions.



FIG. 9.—Unmelted and renovated butter under polarized light.

The above sketches illustrate the appearance of (1) unmelted and, therefore, presumably pure, butter, examined under polarized light, and (2) melted and solidified fat, in light and dark fields, under polarized light. These are presumably either renovated or adulterated butter.

LARD.

At one time, lard was regarded as the fat rendered from specified parts of the hog, but as the fat is now rendered in enormous quantities from other parts than the kidneys and the bowels—which used to furnish the fat known as lard—the term lard has now a rather wider signification.

According to Lewkowitsch, the following grades of lard are recognized in the American packing trade.

(1) Neutral lard No. 1. The fat rendered between 40° to 50° in a perfectly pure state from the leaf (kidneys and bowels). It is practically neutral.

(2) Neutral lard No. 2. This is the fat of the back rendered under the same conditions.

(3) Leaf lard. On subjecting the residue not rendered for neutral lard to steam heat under pressure, the leaf lard of commerce is obtained.

(4) Choice lard. This is defined by the Chicago Board of Trade as made from the leaf and trimmings only, either steam or kettle rendered (i.e. in open steam-jacketed vessels). Neutral lard may have first been rendered.

(5) Prime steam lard. This is made from any part of the hog and is rendered in tanks by the direct application of steam.

(6) A still lower quality is rendered from the whole of the abdominal viscera.

The usual American standards for "standard lard" and "standard leaf lard," are lard, and leaf lard respectively, containing at least 99 per cent of fat (including fatty acids), and having an iodine value not above 60.

Lard consists of the glycerides of lauric, myristic, palmitic, stearic, oleic and linoleic acids. According to Twitchell the composition of the mixed fatty acids of lard is as follows:—

	Per cent
Linoleic acid	10.06
Oleic acid	49.39
Solid fatty acids	40.55

Lewkowitsch has summarized the following comparisons between European lards and American lards made from various portions of the hog ("Oils, Fats, and Waxes," Vol. II, p. 781, 3rd edition).

EUROPEAN LARDS.

Fat from	Sp. gr. 100°/15°.	M. Pt. of Fat.	M. Pt. of Fatty Acids.	Iodine No. of		Free Acids as Oleic.
				Fat.	Fatty Acids.	
				Per cent	Per cent	Per cent
Back	0.8607	33.8°	40°	60.6	61.9	0.152
Kidney	0.8590	43.2°	43.2°	52.6	54.2	0.163
Leaf	0.8588	44.5°	42.9°	53.1	54.4	0.360

NORTH AMERICAN LARDS.

	Specific gravity 100°/15°.	Iodine No.	Melting-point.	Butyro-refractometer No. at 40° C.
		Per cent		Per cent
Head	0.8637	66.2	44.8°	52.6
	0.8629	66.6	44.8°	52.5
	0.8631	65.0	45°	52
Back	0.8611	61.5	48.5°	52.4
	0.8621	65	48.5°	51.8
	0.8616	65.1	46°	51.9
Leaf	0.8637	62.2	45°	51.4
	0.8615	59	44°	50.2
	0.8700	63	44.5°	52
Foot	0.8589	68.8	40°	44.8
	0.8641	68.4	45°	51.9
Ham	0.8615	66.6	44°	51.9
	0.8628	68.3	44.5°	53

The following are the average constants for a large number of genuine lards. The determinations were made in the author's laboratory except when otherwise mentioned :—

LARD.

Sp. gr. at $\frac{100^{\circ}}{15^{\circ}}$.	Melting-point.	Saponification Value.	Iodine No.	Refractive Index at 60° C	Butyro-refractometer No. at 40°.	Observers.
0.859 to 0.862	40 to 47°	194 to 197	54 to 68	1.4527 to 1.4541	—	Parry
— 0.861	—	195 „ 196	53 „ 77	—	—	Lewkowitsch
0.859 „ 0.864	—	—	59 „ 68	—	45 to 53	Dennstedt

FATTY ACIDS FROM LARD.

Sp. gr. at $\frac{100^{\circ}}{15^{\circ}}$.	M. Pt.	Mean Molecular Weight.	Iodine No.	Refractive Index at 60° C.	
0.837 to 0.840	44°	278	—	—	Allen
0.836 „ 0.841	42 to 45°	275	59 to 66	1.439 to 1.441	Parry

Genuine lard should contain under 0.5 per cent of unsaponifiable matter, which is principally cholesterol.

Lard is official in the British Pharmacopœia, the melting-point being given as about 37.8° :—

Lard is adulterated to a considerable extent. Beef fat, cotton-seed stearin and maize oil are the principal adulterants. The so-called compound lard is sometimes a mixture of lard stearin from which the more liquid portion of the oil has been removed and sold as lard oil, with beef stearin and cotton-seed or maize oil. Frequently no lard at all is present.

Interpretation of Analytical Results.—Any lard having a specific gravity at $\frac{100^{\circ}}{15^{\circ}}$ above 0.864 must be regarded with great suspicion, and is probably adulterated. The following table shows the effect of individual adulterants on the specific gravity of lard, but it must be remembered that mixtures are easy to make up which have the same specific gravity as pure lard.

Fat.	Specific gravity at $\frac{100^{\circ}}{15^{\circ}}$.	Observers.
Pure lard	0.859 to 0.862	Parry
Lard stearin	0.857 „ 0.858	Parry
Cotton-seed oil	0.868 „ 0.8725	Allen—Pattinson
Cotton-seed stearin	0.8658 „ 0.8662	Parry
Beef stearin	0.857	Pattinson
Arachis oil	0.8673	Allen
Cocoanut oil	0.8736	Allen

The iodine value must be regarded with caution, as reliable observers have shown that occasionally values well above the generally accepted limits may be found. Lewkowitsch considers that 50 to 66 is a fair range of values, and that outside these figures, a sample should be regarded with suspicion, or at least as inferior lard. The average iodine absorptions for various adulterants are as follows:—

Beef stearin	20
Beef fat	38
Mutton fat	40
Cotton-seed oil	110
Cotton-seed stearin	90
Cocconut stearin	4 to 6

The refractometric examination, like all the other results, must be regarded in conjunction with all the other figures.

According to Mansfeld, Bömer, and Dennstedt and Voigtländer, the following values cover a large number of authentic samples:—

Fat.	Butyro-refractometer No. at 40°.
Lard from back	50·2 to 52·4
„ „ head	52·2 „ 52·6
„ „ leaf	50·2 „ 52
„ outer part of leaf	50·7
„ from belly	50·4
„ „ intestines	49
„ „ foot	44·8
„ „ ham	49·1 „ 53
Beef tallow	49
Horse fat	53·7
Cocconut fat	35·5
Cotton-seed oil	61

Generally speaking a low refractometer number indicates the presence of beef stearin or cocconut fat, whilst a high value indicates cotton-seed stearin.

The deviations in Amagat and Jean's instrument show wider differences still. The following figures are due to Dupont:—

Pure lard	- 12·5°
Lard stearin	- 10 to - 11°
Beef stearin	- 34°
Cotton-seed stearin	+ 25°
Arachis oil	+ 5°
Cocconut oil	- 54°

Vegetable oils may, of course, be indicated by the above tests, examined collectively, but the only certain proof of the presence of a vegetable fat, when the figures have been adjusted by judicious mixtures, is a positive response to the phytosteryl acetate test (p. 630).

If a vegetable oil has been indicated, however, by a high iodine value, the following oils should be looked for:—

Arachis Oil.—This should be searched for by any modification of Renard's test (see olive oil, p. 113). The amount of arachis oil may be calculated from the amount of arachidic acid found.

Sesame Oil.—This is detected by the hydrochloric acid and furfural test (p. 116).

Maize Oil.—There is no direct test for this, but in the absence of arachis, sesame and cotton-seed oils, a high iodine value and a low melting-point of the fatty acids would indicate maize oil.

Cotton-seed Oil or Stearin.—The Becchi and Halphen tests (see p. 115) may be applied, but neither does a negative reaction prove the absence of cotton-seed oil, nor does a positive reaction prove its presence. It has been abundantly proved that lard obtained from hogs fed on cotton-seed cake yields a cotton-seed oil reaction, and as it cannot be suggested that such feeding is objectionable, no great reliance must be placed on these reactions.

If a positive reaction, combined with a high iodine value, be obtained, cotton oil may almost safely be presumed to be present. The final test to decide with certainty is the phytosteryl acetate test referred to above.

A microscopical examination of lard may yield useful results. Five grms. of the fat, free from moisture, should be dissolved in 20 c.c. of a mixture of 90 per cent ether and 10 per cent alcohol and the liquid allowed to stand at about 20° over night. In the event of no crystals forming by the morning, the stopper should be removed from the tube and a plug of cotton wool inserted, so as to allow a slow evaporation of the solvent. If the crystals form rapidly, they must be redissolved and redeposited. The mother liquor is decanted and the crystals examined under the microscope. Such crystals from pure lard usually form oblong plates, either alone or in bunches, cut off obliquely at one end. Beef stearin, on the other hand, forms curved tufts of thin needles, often resembling the letter *f* in shape. The ends are sharp, and the needles are often arranged in fan-shaped clusters. It is necessary to use a high power in order to show the plate-like structures of lard crystals, as under low powers they may appear as needles. Under low powers also, the lard crystals *may* appear curved, but under a high power, this will be found due to the fact that several crystals are joined at various angles. Stock ("Analyst," xix. 2) compares the crystals deposited from ether with those from two standard sets of mixtures, the first consisting of pure lard melting at 34° to 35°, containing 5, 10, 15 and 20 per cent of beef stearin melting at 56°; the second consisting of pure lard melting at 39° to 40°, with 5, 10, 15 and 20 per cent of beef stearin melting at 50°. Three c.c. of the melted fat are mixed with 21 c.c. of ether in a 25 c.c. stoppered cylinder, and the mixture warmed to 20° to 25°. If the sample melts at about 35°, 3 c.c. of each of the series of the first set of standards are treated in exactly the same manner. If the sample melts at about 40°, the second series is employed instead. The cylinders are now cooled down to 13° and kept for twenty-four hours. The ether is decanted and 10 c.c. of pure ether at 13° C. is added in each case. After standing at 13° for twenty-four hours, the contents are poured off into shallow beakers, the ether poured off and the crystals allowed to dry for fifteen minutes at 10° C., and weighed. The approximate amount of adulterant is arrived at by comparing the weight obtained with that of the type sample nearest to it. The crystals should then be examined microscopically in comparison with the type samples.

SUET.

The unrendered fat of various animals, principally oxen and sheep, is known as suet. The corresponding rendered fat, freed from cellular tissue, etc. is known as tallow.

Suet is usually sold as beef suet or mutton suet, and being handled in its natural state gives little opportunity for adulteration.

The principal difference between beef and mutton suet is that mutton suet is richer in stearin than beef suet. The following are the principal characters of beef and mutton suet:—

	Beef Suet.	Mutton Suet.
Specific gravity at 15°	0·942 to 0·953	0·937 to 0·953
" " " ¹⁰⁰ / ₁₅ °	0·860 ,, 0·864	0·858 ,, 0·864
Melting-point	38 ,, 46°	45° ,, 50°
Saponification value	192 ,, 200	192 ,, 198
Iodine value	35 ,, 46	34 ,, 48
Reichert value	0·25	0·25
Hehner value	95 to 96	95 to 96
Refractive index at 60°	1·4510	1·4500
Butyro-refractometer No. at 40°	49	48 to 49
Specific gravity of fatty acids, ¹⁰⁰ / ₁₀₀ °	0·870	0·869 ,, 0·872
Melting-point of fatty acids	43 to 47°	46° ,, 54°
Mean molecular weight of fatty acids	270 ,, 285	266 ,, 275
Refractive index of fatty acids at 60°	1·4375	1·4374

OLIVE OIL.

At one time the names olive oil and salad oil were considered synonyms in the oil trade, but to day, owing to the fact that several other vegetable oils are quite suited for edible purposes, oils sold under the name of salad oil are not necessarily olive oil.

Olive oil is pressed from the fruit of the olive tree, *Olea europaea*. It is true that some of the oil is also extracted by means of a suitable solvent, but the resulting oil is only fit for use for industrial purposes, so that from the present point of view, we need only consider olive oil of the edible type.

There are very numerous species of the olive tree, and this fact, together with the effect of climate, soil and method of cultivation, accounts for the numerous types of genuine olive oil which may be found on the market. The finest oil is obtained from hand-picked fruits, and the olive stones are not crushed in the pressing—the oil from olive kernels is not quite identical with that from the pulp of the fruits. There are various grades, such as the first pressed oil, which is always the best, and the second pressings, which are usually obtained by moistening the marc from the first pressings, and subjecting it to a further pressing. Apart from the question of purity, the essential feature of edible olive oil is that it should contain only a very small amount of free fatty acids: as a rule from 0·2 per cent to 0·5 per cent will be found to cover the best samples. Samples con-

taining more than this cannot be condemned as impure, but must be judged as inferior oils. Commercial oils used for pharmaceutical purposes, such as for the preparation of liniment of camphor, will usually be judged as of good quality when they contain less than 4 per cent of free fatty acids, when calculated as oleic acid.

Olive oil consists of a large quantity of olein, mixed with some stearin, palmitin, and other glycerides. It is a limpid liquid of pale yellow colour, or in the lower grades, sometimes green, but never so in the case of the best oils. Its taste is sweet and bland, recalling that of the fruit. This last feature varies very greatly with the district in which the olives are grown—Tunisian oil, for example, has a rather harsh taste, and for many years there is no doubt that Tunisian oils were improperly rejected by the French official analysts, perhaps partly on this account, but also because they were found to yield a slight reaction then believed to be absolutely characteristic of sesame oil. It is now well established that injustice was done to this oil, and a very large trade in it now exists. It is generally necessary to blend the oil from such districts with sweeter oil from other districts. In choosing an oil for such purposes as tinning sardines, the finest oil alone should be used, as a poor-quality oil will spoil the finest sardines.

In judging the purity of olive oil, the following are the principal features to determine:—

Specific gravity: iodine value: solidifying point: saponification value: the characters of the fatty acids: the refractive index, and special tests for certain oils, which will be mentioned later. The following table is compiled from the examination of about 100 samples of edible olive oils in the author's laboratories, but for the sake of completeness, the figures for ordinary oils, which are rather wider in their limits, are added as well.

CHARACTERS OF OLIVE OIL.

	Edible Oils.	Olive Oil in general.
Specific gravity at 15° C.	0.915 to 0.9175	0.914 to 0.919
Iodine value	82 „ 85	80 „ 88
Solidifying point	+ 1° „ + 3°	+ 1° „ + 4°
Butyro-refractometer No. at 20°	66 „ 67	66 „ 67
Refractive index at 20°	1.4690 „ 1.4671	1.4669 „ 1.4671
Saponification value (°/o KOH)	18.9 „ 19.1	18.5 „ 19.5
Reichert value	0.2 „ 0.3	0.2 „ 0.3
Maumené test (with H ₂ SO ₄)	41° „ 46°	41° „ 46°
Melting-point of insoluble fatty acids	24° „ 25.5°	24° „ 27°
Mean molecular weight of „	280 „ 286	280 „ 286
Free fatty acids	0.1 „ 1.5 %	—

Olive oil is considerably adulterated. At one time the favourite adulterant was poppy-seed oil, but this has long given way to other oils of which the favourites are arachis, sesame and cotton-seed oils.

It will not be necessary to deal here with the adulteration with such oils as castor and rape, as these are only found in industrial oils, their taste preventing them from being employed for admixture with edible oils.

The effects on the analytical results, of the principal adulterants are as follows.

(1) *Arachis Oil*.—The specific gravity of this oil is slightly higher than that of olive oil, but 30 to 40 per cent may be added without raising this figure beyond that of normal olive oil. The iodine value is about 95 to 98, so that a substantial addition of arachis oil *may* be indicated by a high iodine value. As the fatty acids melt at 31° to 32°, a slightly higher melting-point may be expected if much arachis oil is present. The action of nitrous acid on arachis oil differs from that on olive oil, and may roughly indicate the presence of this or another adulterant. Olive oil yields the hardest elaidin of all oils, and much arachis oil will prevent the formation of more than a buttery mass except after a long time. The elaidin test is best carried out as follows. Ten c.c. of the oil are mixed with 5 c.c. of nitric acid (specific gravity 1.4) and 1 grm. of mercury, and shaken till the mercury dissolves. It is then allowed to stand for twenty minutes, and then again shaken for one minute. Olive oil yields the hardest mass as a result, but arachis oil yields a hard elaidin, only in a rather longer time, so that nothing more than general indications are yielded by this test, unless a very soft elaidin, such as would be due to the presence of a large quantity of sesame oil, results, when a very strong inference of adulteration may be drawn. The decisive test for arachis oil, however, is the determination of the arachidic acid. The very small quantity of arachidin present in olive oil does not interfere with the approximate accuracy of the process. But it must be remembered that the process is only approximate, since arachis oil contains a variable amount of arachidic acid, so that an average factor must be used to convert the arachidic acid into arachis oil. Reliable analysts have isolated from 4.5 per cent to 5 per cent (Renard); 4.37 per cent to 4.8 per cent (De Negri and Fabris); 5.5 per cent (Allen); 4.31 per cent to 5.4 per cent (Tortelli and Ruggeri). The usually adopted factor is based on an average value of 5 per cent, and this will give results as near as are usually necessary. If the arachidic acid be purified so as to melt at 74° to 75°, 4.8 is a more accurate factor. The process is carried out as follows (this was originally described by Renard, "Comptes Rendus," **73**, 1330): 10 grms. of oil are saponified with alcoholic potash, and the fatty acids separated in the usual manner, by driving off the alcohol, and acidifying the aqueous solution with hydrochloric acid. The fatty acids are washed, and dissolved in 90 per cent alcohol. Excess of a solution of lead acetate is now added. The precipitated lead salts are filtered off, washed with alcohol, and dried. They are then extracted in a Soxhlet tube with ether so as to separate the lead salts of the unsaturated and saturated fatty acids. The lead salts of the saturated fatty acids remain undissolved, and are decomposed by the addition of hydrochloric acid under ether: the latter dissolves the fatty acids, and is separated, the

residue washed with ether and the whole of the solvent evaporated, leaving the residue of fatty acids. This is dissolved in 50 c.c. of hot 90 per cent spirit, transferred to a covered vessel, and allowed to cool. If any quantity of arachis oil be present a crop of crystals of arachidic acid will be formed when the alcohol has cooled. If this be the case, filter off the crystals, wash the filter with 90 per cent and then with 70 per cent alcohol, noting the quantity of 90 per cent alcohol used for washing. Now dissolve the crystals by pouring boiling absolute alcohol through the filter, evaporating the solvent in a small capsule and weighing the residue. To the weight so found, add 0.0022 gm. for each 10 c.c. of 90 per cent alcohol used for washing at 15° C., or 0.0045 gm. if at 20° C. This crude arachidic acid, multiplied by 20, gives approximately the amount of arachis oil present in the sample. Its melting-point should be 71° to 72°.

There are various modifications of this process. Lewkowitsch prefers to neutralize the saponification liquids with acetic acid, using phenol-phthalein as an indicator, and then precipitate with lead acetate, in order to save the trouble of separating the fatty acids. Tortelli and Ruggeri prefer to use 20 grms. of oil and to dissolve the fatty acids liberated from the lead salts not dissolved by the ether extraction, in 100 c.c. of 90 per cent alcohol, on the water bath at about 60° C. A drop of hydrochloric acid may be added if the liquid is turbid. The liquid is allowed to stand for three hours at 15° or 20°. The separated fatty acids are transferred to a filter (the filtered liquid may be used to assist in this operation). They are then washed thrice with 10 c.c. of 90 per cent alcohol, and then with 70 per cent alcohol. The crystals are now dissolved in boiling absolute alcohol. The solvent is driven off, and the residue again dissolved in 100 c.c. of 90 per cent alcohol, and the separation and filtration and washing carried out as before. The residue is now dissolved in absolute alcohol again, the solvent driven off, and the residue weighed. The crystals should now melt at 74 to 75.5°. The following table (from Lewkowitsch) gives the amount to be added to the weight found, due to the solubility in 90 per cent alcohol according to Tortelli:—

100 C.C. OF 90 PER CENT ALCOHOL DISSOLVE ARACHIDIC ACID MELTING AT 74-75°.

Amount of Acid found.	At 15°.	At 17.5°.	At 20°.
2.7 gr. down to 0.5 gr.	0.070 gr.	0.080	0.090
0.5 " " 0.17 "	0.050	0.060	0.070
0.17 " " 0.05 "	0.033	0.040	0.045

The following qualitative method will detect 10 per cent of arachis oil. Saponify 1 c.c. of the oil with 5 c.c. of an 8.5 per cent solution of ordinary caustic potash in absolute alcohol. This will only take a few minutes. Exactly neutralise with 90 per cent acetic acid, and then add 50 c.c. of 70 per cent alcohol containing 1 per cent of ordinary

laboratory hydrochloric acid. Cool to 18° to 20°. In the presence of 10 per cent, often as low as 5 per cent, of nut oil—crystals of arachidic acid will separate. In general pure olive oil will remain quite clear, but occasionally a sample will give a slight flocculent deposit easily distinguished from arachidic acid.

The French Codex prescribes the following test:—

One c.c. of olive oil and 15 c.c. of alcoholic potassium hydroxide solution (5 per cent) are boiled for twenty minutes in a small flask under a reflux condenser. The liquid is then to be kept in a cool place for twelve hours, at the end of which it should still be limpid.

(2) *Cotton-seed Oil*.—The specific gravity of cotton-seed oil is about 0.922 so that appreciable quantities would slightly raise this figure, although moderate quantities might not affect it appreciably. The iodine value is about 110, so that a high iodine value for any sample of olive oil would suggest the presence of cotton oil. The most valuable quantitative process, however, is the determination of the iodine value of the liquid fatty acids, which have—in the case of cotton and olive oils—a far greater difference than the oils themselves. The liquid fatty acids are prepared by converting the fatty acids into lead salts, extracting the lead salts of the liquid fatty acids by ether (as described in the previous paragraph) and decomposing the lead salts in the usual manner. The iodine value of the liquid fatty acids of pure olive oil varies from 94 to 96.5. That of the liquid fatty acids of cotton-seed oil is from 145 to 150, so that the presence of 10 per cent of cotton-seed oil is clearly indicated.

Several colour reactions have been recommended, and are to some extent trustworthy, but not altogether so. The principal of these tests are those known as the Becchi and the Halphen tests.

Becchi's test as originally introduced involves the use of an alcoholic solution of silver nitrate, and a solvent consisting of amyl alcohol and colza oil, the use of which is far from clear. The test has undergone so many modifications, and been condemned by so many chemists, yet approved by so many others, that a strong difference of opinion exists in regard to it. The simplest and probably most useful form is that of the British Pharmacopœia. It is as follows: If 10 c.c. of the oil be shaken with 2 c.c. of a reagent prepared by dissolving 1 grm. of silver nitrate in 100 c.c. of absolute alcohol, with the addition of 20 c.c. of ether and one drop of nitric acid, no blackening should take place when the mixture is heated on a water bath for ten minutes. There is no doubt that many pure olive oils give a brown or even slight black colour, so that the test is deceptive, but Milliau claims that if the test be applied to the fatty acids instead of to the oil itself, no pure olive oil gives the reaction. Hohner & Lewkowitsch see no advantage in Milliau's suggestion, but the author finds that the results are more trustworthy than on the oils themselves. The Halphen test is the better of the two. It consists in heating in a water bath about 2 c.c. of the oil, with an equal volume of amyl alcohol, and an equal volume of a 1 per cent solution of sulphur in carbon bisulphide. In the presence of cotton-seed oil the mixture becomes of a red colour, its intensity depending on the amount of cotton oil present, in from 5 to 30 minutes. Definite reactions can be obtained with 2 per cent of

cotton oil, and according to Lewkowitsch, even with 1 per cent. It must be remembered, however, that there are certain other oils which give a similar reaction, and also that if cotton-seed oil be heated sufficiently, the substance which is responsible for the Halphen and the Becchi reactions is destroyed, and no reactions will be obtained. These colour tests, therefore, must only be considered as of an indicative or confirmatory nature, and must not be too greatly relied on, unless confirmed by the iodine determination above mentioned.

(3) *Sesame Oil*.—The specific gravity of this oil is about 0.923 to 0.924, and the iodine value about 105. Only faint differences in these figures would therefore be produced by the addition of substantial quantities of sesame oil, although large quantities might be distinctly indicated.

The iodine value of the liquid fatty acids (see p. 115) is from 130 to 140, so that better indications are given by this than by the iodine value of the oils themselves.

There is, however, a colour test which appears to be absolutely reliable for this oil. It is known as Badouin's test (modified in various methods). It consists in dissolving 0.1 gm. of sugar in 10 c.c. of hydrochloric acid of specific gravity 1.19, and adding 20 c.c. of the oil to be tested, and shaking for at least a minute in a stoppered test tube, and allowing the mixture to stand and separate. In the presence of from 1 per cent to 2 per cent of sesame oil a distinct crimson colour will result in the aqueous layer. This reaction appears to be due to the formation of furfural by the action of the acid on the sugar, and Villavecchia recommends the following as the best modification of the test, which it certainly is. Use 0.1 c.c. of a 2 per cent solution of furfural in alcohol, 10 c.c. of the oil, and 10 c.c. of hydrochloric acid. This will certainly reveal the presence of 1 per cent to 2 per cent of sesame oil. It must be noted that some olive oils, especially Tunisian oils, give a slight reaction, but according to Milliau, this is never the case with the fatty acids, so that in doubtful cases the fatty acids should be tested instead of the oil. There is also another point to be remembered. There are many factories where arachis and sesame oils are prepared together. The press cloths are not changed, and after a pressing of sesame oil, the first pressing of arachis oil contains a trace of sesame oil, and the author has examined many samples having every characteristic of pure arachis oil, which could not have contained more than a trace of sesame oil, but which yielded this reaction. Hence a faint trace of sesame oil may be accompanied by a large amount of arachis oil, where such an oil has been used as the adulterant.

(4) *Poppy-seed Oil*.—This is not now a common adulterant for edible oils, but it will be indicated by a rise in specific gravity, and iodine value (which are about .926 and 134 respectively for poppy-seed oil).

The requirements of the British Pharmacopœia for olive oil are as follows: It is a pale yellow or greenish-yellow oil with a faint odour and a bland taste. Specific gravity at 15.5° from 0.914 to 0.919. At 10° it is liable to become of a pasty consistence, and at 0° to form a nearly solid granular mass. It must not yield a black colour when subjected to the silver nitrate test described above.

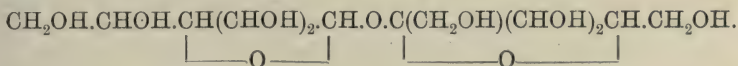
CHAPTER III.

THE CARBOHYDRATE FOODS.

THE carbohydrate foods fall naturally into two groups, the sugars and the starches. Of these the sugars are far better understood from a chemical point of view, the starchy substances being closely related to them, but of a less definite nature. The sugars will therefore be first considered.

Cane Sugar.—The natural sugars which are, or enter into the composition of, food stuffs, are for the most part substitutive derivatives of the hydrocarbon hexane C_6H_{14} . The principal member of the series is cane sugar—known also as sucrose, and giving the generic name of saccharoses to that group of carbohydrates which includes itself, maltose and lactose. Cane sugar forms hard, transparent crystals melting at 160° . After melting it remains for a long time in a transparent amorphous condition (barley sugar), and at higher temperatures it becomes converted into a dark brown amorphous substance known as caramel. Caramel is a mixture of various decomposition products of the sugar.

The formula of sugar is $C_{12}H_{22}O_{11}$, and it is known—as are lactose and maltose—as a twelve-carbon sugar or hexabiose (or saccharose). On hydrolysis cane sugar yields dextrose and levulose (see below) in equal amounts. It can be prepared artificially by the action of acetyl-chlorohydrate on potassium-levulose in alcoholic solution, and undoubtedly is an oxygen ether of the anhydrides of dextrose and levulose, of the constitution



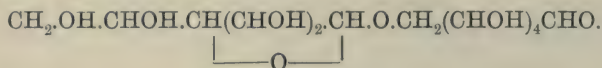
This constitution explains why cane sugar neither reduces alkaline copper solution, nor forms an osazone—both of which positive characters are typical of, and extremely useful in identifying, the sugars which have an aldehydic constitution.

Cane sugar is extracted from the juice of the sugar cane, the sugar beet, the sugar maple, and the sorghum plant. It occurs in numerous other fruits, often associated with other forms of "sugar," but the sugar of commerce is obtained from the three sources above mentioned, cane sugar and beet sugar being used in Europe, whilst these and some maple sugar are employed in America, where the sugar maple flourishes. Loaf sugar is a product which has been rapidly cry-

stallized from hot solutions of the cruder sugar which have been decolorized by animal charcoal. Without this treatment, the crystals will be the cruder brown sugar. Sugar candy is the result of slow crystallization from cold syrup, with a nucleus of string on which the crystals are deposited. Cane sugar is strongly dextrorotatory, the specific rotatory power for the sodium line being $+ 66^\circ$ (the optical properties of sugars will be referred to later). It is soluble in about half its weight of cold water—forming the viscous liquid known as “syrup”. It is nearly insoluble in absolute alcohol, but is readily soluble in dilute alcohol, even in 90 per cent “spirits of wine”; but insoluble in ether and similar liquids. It is oxidized by nitric acid, in the cold slowly, and if the temperature be kept below 50°C. , the product is entirely saccharic acid $\text{C}_6\text{H}_{10}\text{O}_8$, but at 100° oxalic acid is the principal product. Cane sugar is distinguished from the glucoses by the fact that solutions of caustic alkalies have no immediate perceptible action on it, whilst this is not so with the glucoses.

By the action of yeast cane sugar is first transformed into a mixture of dextrose and levulose (invert sugar) which are ultimately further changed by fermentation into alcohol, carbon dioxide and traces of other compounds. Maltose and lactose may now conveniently be shortly described.

Maltose $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$ exists in malt, and of course malt extract, and results, together with dextrin, by the limited action of dilute acids or infusion of malt, which contains the ferment diastase, on starch. It is strongly dextrorotatory, $[\alpha]_d = + 138^\circ$. Although isomeric with sucrose, its constitution is very different, as it is of an aldehydic nature, reducing alkaline copper solution and forming an osazone. Chemically it is glucose-glucoside, of the constitution



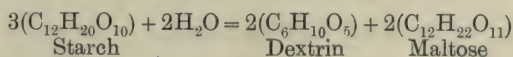
and is stereo-isomeric with lactose. It yields dextrose only on hydrolysis, and is directly fermentable by yeast, if its action be long continued, since yeast usually contains a hydrolysing ferment which causes dextrose to be formed, which is then directly fermented.

It forms fine crystalline needles, which contain one molecule of water of crystallization, which is lost at 100° . It is less soluble in alcohol than sucrose. Maltose exhibits the phenomenon known as bi-rotation, that is, the rotatory power of a freshly made solution is less than that of one which has been kept for some time or has been heated. A cold solution of maltose takes several hours before it attains its full optical activity—a fact of great importance in practice, as will be seen in the sequel. Lactose and dextrose also exhibit bi-rotation, but in these cases the rotatory power diminishes on keeping.

Maltose is hydrolysed by heating with dilute mineral acids, the resulting product being dextrose, the solution increasing in its power of reducing copper solutions, and decreasing in optical activity. Three to four hours boiling with dilute acid is necessary for complete inversion. Maltose is not inverted by the ferments diastase or invertase.

It resembles the glucoses, as mentioned above, in its power of reducing copper salts (as Fehling's solution, *vide infra*). But its reducing power is considerably lower than that of the glucoses, which is easily understood by an inspection of the constitutional formulæ of the compounds—the reducing power depending on the aldehydic complex in the molecule.

Maltose in a more or less pure condition is manufactured by the action of malt infusion on starch. Dextrin in some variety or other is always found at the same time, the proportion varying with the conditions of the reaction. The normal reaction may be represented as follows:—



Lactose or milk sugar, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$, is the sugar found in mammalian milk, in which it is present to the extent of about 5 per cent. It forms hard white rhombic crystals containing one molecule of water, but usually occurs in commerce as a powder, in which form it is largely employed as a constituent of infant foods. It is far less soluble and less sweet than sucrose. It melts, when anhydrous, at 205° and has a specific rotation of $+52.7^\circ$. It reduces metallic solutions, sometimes even in the cold, and forms an osazone. It is not capable of direct fermentation by yeast, but is converted by the lactic ferment into lactic acid. It is hydrolysed by dilute mineral acids yielding equal quantities of glucose and its isomer galactose. It is a stereo-isomer of maltose, and may be chemically described as galactose-glucoside.

A freshly prepared saturated solution contains 14.55 per cent of $\text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_2\text{O}$, but after standing for some time 21.64 per cent is dissolved. This phenomenon has some connexion with that of bi-rotation, for the specific rotatory powers of the two modifications which may be assumed to exist here, are in inverse ratio to the solubility. The least insoluble modification has a specific rotatory power $+80^\circ$ whilst that of the more stable modification is $+52.7^\circ$. In dealing with solutions of lactose, therefore, this fact should be borne in mind.

There are other hexabioses, but they are not of importance from the present point of view.

The six-carbon sugars, or hexoses, to which consideration must now be given are dextrose (which is generally known as glucose), levulose and, incidentally, galactose.

Dextrose, glucose or "starch sugar," $\text{C}_6\text{H}_{12}\text{O}_6$, is a colourless crystalline substance, usually crystallizing with one molecule of water, but can be obtained in an anhydrous condition by crystallization from hot methyl alcohol. It loses its water by crystallization below 100°C. , and when anhydrous melts at 146° . It is soluble in a little more than its own volume of water, forming a syrup which is much less sweet than cane sugar syrup. It exists—with levulose—in honey, and in many fruits, such as the grape, which often contains 15 per cent. It results from the decomposition of many of the glucosides, and is artificially prepared by the hydrolysis of starch or cane sugar. The glucose of

commerce is nearly always the product of the hydrolysis of starch. Some discrepancies exist in the figures usually quoted for the specific rotatory power of dextrose but the probable value is $+53^\circ$. When partly dissolved its rotatory power is much greater, owing to the initial formation of a labile isomeride. The rotation decreases slowly if the solution be left, or rapidly by heating or the addition of alkali, until its permanent value is attained.

Dextrose is soluble in alcohol, is not charred by cold sulphuric acid, and is coloured brown when warmed with caustic soda solution. It has a strong reducing power on metallic solutions such as Fehling's solution, and yields a characteristic osazone. It is directly fermentable by yeast.

The constitution of dextrose is important, as indicating the power of reducing metallic solutions, and of forming an osazone, characters which are always concomitant. It is at once a pentatomic alcohol, containing an aldehyde group, known as an aldose or aldohexose of the constitution $\text{CH}_2\text{OH} \cdot (\text{CHOH})_4 \cdot \text{CHO}$. There is now no doubt that dextrose is in reality a mixture of two bodies of this constitution, differing only in stereochemical relationships. In general, dextrose which has been prepared from cold solutions contains excess of α -dextrose, of specific rotation $+105^\circ$, whereas that separated from solutions that have been heated contains most β -dextrose of specific rotation $+22^\circ$. All forms of dextrose when in solution in water gradually attain a state of equilibrium in which there is nearly half of each form present, hence the fact that ultimately a specific rotation of $+53^\circ$ is attained. It may be mentioned incidentally that dextrose exists, on account of the atomic groupings it contains, in the laevorotatory and optically inactive forms.

Levulose or fructose (fruit sugar) is a laevorotatory six-carbon sugar, containing a ketonic grouping, and is classified as a keto-hexose, $\text{C}_6\text{H}_{12}\text{O}_6$.

It occurs in honey and in various fruits, and is formed in equal amount with dextrose in the hydrolysis of cane sugar. It is also easily obtained by the hydrolysis of inulin, a starchy matter found in dahlia tubers, which yields levulose in the same manner as ordinary starch yields dextrose. It is a colourless crystalline substance, melting at 95° . Its specific rotation is -98.8° at 15° decreasing by 0.6385° for each degree until at 87°C , it is -53° , which is identical with that of dextrose, but opposite in sign. Being a keto-alcohol, it forms an osazone and easily reduces metallic solutions.

The product of hydrolysis of cane sugar is a mixture in equal quantity of dextrose and levulose, which is known as invert sugar. But as the rotation of levulose is higher than that of dextrose, cane sugar is converted by hydrolysis from a dextrorotatory to a laevorotatory substance.

Levulose has the constitutional formula



which indicates its power of forming an osazone and of reducing metallic solutions.

It is of great interest to note that levulose or fructose can be obtained from glucose or dextrose by means of its osazone; and in spite of its levorotation, the name *D*-fructose is retained for levulose in the scientific nomenclature of the sugars, as indicating its genetic relationships. It may also be here mentioned that numerous space isomerides of most of the sugars may, and do, exist, but these the analyst has never to deal with.

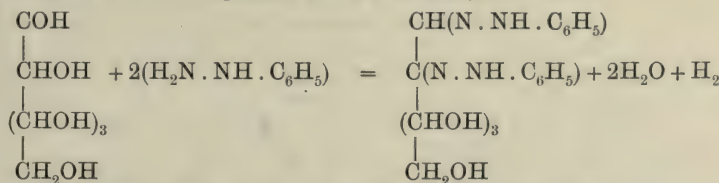
Levulose is separated from dextrose in the reaction products of the hydrolysis of cane sugar by mixing the liquid with powdered slaked lime, in a vessel surrounded by ice. The levulose forms a nearly insoluble compound with calcium, whilst the dextrose compound is soluble and can be filtered off. The levulose compound is decomposed by shaking with oxalic acid, or better, by a current of CO_2 when the filtered liquid yields anhydrous levulose by evaporation in vacuo over sulphuric acid.

Invert Sugar.—This name is given to the mixture of dextrose and levulose, either occurring naturally, when it may have resulted from the hydrolysis of cane sugar (natural invert sugar has not a constant composition, as the conditions of its formation are not constant); or prepared artificially, especially for brewers' use, and sold under the names of "invert sugar," "saccharum," or "saccharine".

Other compounds of this group will be referred to as found necessary under special paragraphs.

THE CHARACTERISTICS OF THE SUGARS.

The Phenyl-hydrazine Compounds.—The hexoses, of which dextrose and levulose are typical (as also galactose the product, together with dextrose, of the hydrolysis of lactose) show the reactions of alcohols, and those of aldehydes or ketones. Of the hexabioses under consideration, cane sugar contains no aldehydic or ketonic groupings, whilst maltose and lactose both possess aldehydic functions. Those sugars, then, which contain aldehydic or ketonic groupings, are capable of reacting with phenyl-hydrazine, and in some cases of forming compounds which are well suited to characterize the several sugars. In general, the sugars, in the presence of excess of phenyl-hydrazine $\text{C}_6\text{H}_5 \cdot \text{NH} \cdot \text{NH}_2$, react, with the formation of osazones, in accordance with the equation (for the hexoses),



The reaction is carried out by adding two parts of phenyl-hydrazine and two parts of 50 per cent acetic acid, to about 1 part of glucose or other sugar in 20 parts of water. The mixture is digested for about an hour on the water bath, when the osazone will separate on cooling.

Dextrose and levulose yield the same glucosazone under these circumstances, which clearly indicates the identity of the CH_2OH (CHOH)₃ grouping in both compounds as will be seen from an inspection of the above equation. The crystalline osazone of dextrose or levulose is collected on a filter, washed with a little water and dried. To ensure absolute purity, it may be recrystallized from hot alcohol. It is then found to be a golden yellow crystalline powder melting at 204° . The formation of this compound is absolutely indicative of the presence of sugar in diabetic urine, where substances may occur which simulate the other reactions of glucose. The osazone of lactose, produced under similar conditions, melts at 200° , and that of maltose at 206° . If the osazones are sufficiently well purified, these melting-points are sharp, and quite characteristic (especially when taken in conjunction with the other properties of the sugars) of the several individuals.

THE SUGARS AS REDUCING AGENTS.

The majority of sugars are either aldehydic or ketonic in character, and as such, possess greater or less power of acting as reducing agents. Those sugars which are not aldehydic or ketonic in character, and do not form phenyl-hydrazine compounds, do not reduce metallic solutions—(or if so only very slightly and with difficulty).

In alkaline solutions, the aldehyde and ketone sugars reduce picric acid to picramic acid; indigotin to indigo white; and ferricyanides to ferrocyanides. Bismuth, mercury, silver, gold and platinum salts are reduced to the metallic state; and ferric and cupric salts to ferrous and cuprous compounds.

In practice, the reduction of cupric salts to cuprous oxide is almost universally used as a quantitative method for the determination of the sugars. The use of mercuric salts is not uncommon, but other reductive processes are rarely used. These, then, are the only reduction processes, which need be described in any detail:—

(1) *Copper Salts*.—Many organic compounds prevent the precipitation of cupric sulphate by caustic soda or potash. The best compound for preventing such precipitation is tartaric acid or a soluble tartrate. If a solution of cupric sulphate, caustic soda and sodium tartrate be made, it can be boiled without any precipitation occurring, but in the presence of a *reducing* sugar a yellowish-red precipitate of cuprous oxide occurs, and if it be present in sufficient quantity the blue colour of the solution entirely disappears, the whole of the copper being precipitated in the form of cuprous oxide. This reaction has been utilized in numerous ways as the basis of a quantitative determination of various sugars.

The most generally employed method is the volumetric process with Fehling's solution. To prepare this, 34.64 grms. of pure crystalline cupric sulphate is dissolved in distilled water to form 500 c.c. This is labelled No. 1 solution. No. 2 solution is prepared by dissolving about 70 grms. of caustic soda (as nearly pure as possible) and 180 grms. of potassium sodium tartrate, in water to form 500 c.c. These

solutions should be kept separate, and mixed in exactly equal volumes as necessary, the mixture then forming Fehling's solution. This, when boiled, should remain quite clear. If the two solutions are kept mixed for any length of time no reliance should be placed on the mixture for quantitative results.

To detect a reducing sugar by means of Fehling's solution, the liquid to be tested must be clear and nearly colourless. If the solution is dark coloured it should be clarified by the addition of lead acetate solution, the excess of lead removed by a strong solution of sulphurous acid, and a little washed moist alumina added. The liquid is made up to a definite volume and filtered. With dark coloured liquids qualitative reactions are difficult, and quantitative reactions impossible.

If a solution containing a reducing sugar be boiled with Fehling's solution, a yellow, or orange red, precipitate of cuprous oxide is formed. The hexoses, maltose and lactose reduce Fehling's solution, but sucrose does not do so until after inversion. There are other substances which reduce Fehling's solution, but, except in the case of urine analysis, these do not as a rule give any difficulty in practice. In doubtful cases, recourse must be made to the phenyl-hydrazine test.

As a quantitative reaction, the reduction of copper salts may be used gravimetrically or volumetrically. The latter is the more generally used method.

In this process, in which Fehling's solution is used, the saccharine solution containing, as nearly as can be judged, about 1 per cent of sugar, is placed in a burette, and 10 c.c. of Fehling's solution is placed in a white porcelain basin with 30 c.c. of water, over a Bunsen burner. When the solution is boiling the sugar solution is run in 2 c.c. at a time at first, with boiling after every addition. As the cuprous oxide is precipitated, the blue gradually lessens and as it is nearly gone, the sugar solution is added more cautiously, but as rapidly as possible. The end of the reaction is noted by allowing the precipitate to settle, and noting that the blue colour has entirely disappeared, leaving the supernatant liquid colourless or faintly yellow. Or a drop or two of the liquid may be rapidly filtered through a little glass wool and spotted with a mixture of acetic acid and potassium ferrocyanide on a white tile, when excess of copper will produce a brown coloration.

As determined in the foregoing manner, 10 c.c. of Fehling's solution correspond to the following weights of sugar (these figures are the average of numerous determinations by various observers :—

Dextrose or levulose (and invert sugar)	0.0505	gm.
Lactose	0.0685	"
Maltose	0.0810	"
Sucrose, after inversion	0.0475	"

In the determination of sucrose, it should be inverted by heating 50 c.c. of the saccharine liquid with 5 c.c. of strong hydrochloric acid slowly to 68° C. and then allowing it to cool. If coloured, the liquid may be treated with a little animal charcoal and filtered, or, if necessary, clarified by treatment with lead as described above. Excess of acid should be neutralized by sodium carbonate before the addition of Fehling's solution.

Fehling's solution should be, for exact work, standardized against pure cane sugar: but if very exact results are required, the gravimetric process must be employed.

The following are the details of the gravimetric process, using Fehling's solution:—

About 50 c.c. of Fehling's solution is diluted with an equal volume of boiling water that has been well boiled in order to expel dissolved oxygen. The liquid is kept in a small beaker immersed in a second beaker in which the water is kept boiling. When the temperature of the diluted Fehling's solution is nearly 100° C., a known volume of the sugar-containing liquid is added (previously neutralized, if necessary) and the mixture kept in the boiling water for twelve to fifteen minutes. If the amount of sugar present is large, the blue colour will soon disappear, and a further quantity of Fehling's solution should at once be added. The precipitated copper oxide is rapidly collected on a double filter paper, washed with boiling, well boiled, water, dried, ignited for five or six minutes in an open crucible and weighed as CuO. As cupric oxide is hygroscopic, it must be kept in a desiccator and rapidly weighed. A. H. Allen gives the following figures as the equivalent amounts of sugars corresponding to 1 gram of cupric oxide:—

Glucose.	Cane Sugar (inverted).	Milk Sugar.	Maltose.
0.4535 gr.	0.4308 gr.	0.6153 gr.	0.7314 gr.

O'Sullivan introduced the symbols K and R as indicating the relative reducing values of carbohydrate mixtures, referred to dextrose and maltose respectively. If the K value of dextrose be taken as 100, a substance of half the reducing power of dextrose will have K = 50. In the same way R is taken as 100 for the reducing value of maltose.

The following factors may be employed for the approximate calculation of the principal sugars from the weight of copper or copper oxide obtained:—

	Glucose.	Cane Sugar (after inversion).	Milk Sugar.	Maltose.
Copper	0.5634	0.5395	0.7707	0.9039
Cuprous oxide	0.5042	0.4790	0.6843	0.8132
Cupric oxide	0.4535	0.4308	0.6153	0.7314

Thus if 0.2 grm. of copper has been obtained 0.2×0.5395 will give the equivalent of cane sugar.

These factors are not absolutely correct, especially for certain values, and various tables from which the amount of dextrose can be shown at once, have been constructed. The following is probably the most accurate of such tables:—

Copper. Mg.	Dextrose. Mg.	Copper. Mg.	Dextrose. Mg.	Copper. Mg.	Dextrose. Mg.	Copper. Mg.	Dextrose. Mg.	Copper. Mg.	Dextrose. Mg.
10	5.7	62	31.8	114	57.3	166	83.7	218	111.1
11	6.2	63	32.3	115	57.8	167	84.2	219	111.6
12	6.8	64	32.8	116	58.3	168	84.7	220	112.2
13	7.2	65	33.3	117	58.8	169	85.2	221	112.7
14	7.8	66	33.8	118	59.3	170	85.7	222	113.2
15	8.6	67	34.3	119	59.8	171	86.3	223	113.7
16	9.0	68	34.9	120	60.2	172	86.8	224	114.3
17	9.5	69	35.4	121	60.7	173	87.3	225	114.8
18	10.0	70	35.9	122	61.2	174	87.8	226	115.4
19	10.5	71	36.4	123	61.7	175	88.3	227	115.9
20	11.0	72	36.8	124	62.2	176	88.9	228	116.4
21	11.6	73	37.3	125	62.8	177	89.4	229	117.0
22	12.0	74	37.8	126	63.3	178	89.9	230	117.5
23	12.5	75	38.3	127	63.8	179	90.4	231	118.1
24	13.0	76	38.6	128	64.3	180	91.0	232	118.6
25	13.5	77	39.0	129	64.8	181	91.5	233	119.2
26	14.0	78	39.4	130	65.3	182	92.0	234	119.7
27	14.5	79	40.0	131	65.8	183	92.5	235	120.3
28	15.0	80	40.5	132	66.3	184	93.1	236	120.8
29	15.5	81	41.0	133	66.8	185	93.6	237	121.3
30	16.0	82	41.5	134	67.3	186	94.1	238	121.8
31	16.5	83	42.0	135	67.8	187	94.6	239	122.4
32	17.0	84	42.5	136	68.3	188	95.1	240	122.9
33	17.5	85	42.9	137	68.8	189	95.7	241	123.5
34	18.0	86	43.4	138	69.4	190	96.2	242	124.0
35	18.5	87	43.9	139	69.9	191	96.7	243	124.6
36	19.0	88	44.4	140	70.4	192	97.2	244	125.1
37	19.5	89	44.9	141	70.9	193	97.7	245	125.7
38	20.0	90	45.4	142	71.4	194	98.3	246	126.2
39	20.4	91	45.9	143	71.9	195	98.8	247	126.8
40	20.9	92	46.4	144	72.4	196	99.3	248	127.3
41	21.4	93	46.8	145	72.9	197	99.8	249	127.9
42	21.9	94	47.3	146	73.4	198	100.4	250	128.4
43	22.4	95	47.8	147	73.9	199	100.9	251	128.9
44	22.9	96	48.3	148	74.5	200	101.4	252	129.4
45	23.4	97	48.8	149	75.0	201	101.9	253	130.0
46	23.9	98	49.3	150	75.5	202	102.5	254	130.6
47	24.4	99	49.8	151	76.0	203	103.1	255	131.1
48	24.9	100	50.3	152	76.5	204	103.6	256	131.7
49	25.4	101	50.8	153	77.0	205	104.1	257	132.2
50	25.9	102	51.3	154	77.5	206	104.6	258	132.8
51	26.4	103	51.8	155	78.0	207	105.2	259	133.3
52	26.9	104	52.3	156	78.5	208	105.7	260	133.9
53	27.4	105	52.8	157	79.0	209	106.2		
54	28	106	53.3	158	79.6	210	106.7		
55	28.4	107	53.8	159	80.1	211	107.3		
56	28.9	108	54.3	160	80.6	212	107.8		
57	29.3	109	54.8	161	81.1	213	108.4		
58	29.8	110	55.3	162	81.6	214	108.9		
59	30.3	111	55.8	163	82.1	215	109.4		
60	30.8	112	56.3	164	82.6	216	109.9		
61	31.3	113	56.8	165	83.2	217	110.5		

Pavy's Method.—Dr. Pavy has introduced a useful modification of Fehling's process for determining sugar. It has the advantage of yielding a sharp end reaction, and depends on the fact that in the presence of excess of ammonia, the cuprous oxide is not precipitated but forms a colourless solution. This solution is extremely easy to oxidize, therefore contact with the air must be avoided. The ammoniacal solution is prepared by adding 300 c.c. of strong ammonia solution (0.880 specific gravity) and 400 c.c. of a 12 per cent solution of caustic soda, to 120 c.c. of ordinary Fehling's solution, and making up to one litre with distilled water. One hundred c.c. of this solution has the same oxidizing power on dextrose as 10 c.c. of the ordinary Fehling's solution, i.e. it corresponds to 0.050 gm. of dextrose. The determination is carried out by introducing 100 c.c. of the copper solution into a wide-mouthed flask having an india-rubber cork with two perforations. The nose of the burette containing the sugar solution is passed through one of these, and a bent tube to carry over steam and ammonia vapour is passed through the other. A few fragments of well-burnt pumice are added and the liquid boiled; the sugar solution is then run in, boiling well after each addition, when the blue colour fades and finally disappears. Hehner has shown ("Analyst," vi. 218) that the presence of varying amounts of salts, such as alkaline tartrate or carbonate affects the accuracy of the process considerably. The oxidizing power of this solution is only $\frac{5}{8}$ of that of the ordinary Fehling's solution on dextrose, levulose or invert sugar. Hence the fact that 120 c.c. are diluted to a litre instead of 100 c.c. The reducing action of lactose and maltose on Pavy's solution is not identical with that on Fehling's solution, and reliable figures for these sugars are wanting. It is to be noted that the process of reduction is slower with this solution than with Fehling's, hence longer boiling is necessary.

GERRARD'S PROCESS.

The formation of a colourless double cyanide of potassium and copper is the basis of a method devised by A. W. Gerrard. He prepares the following three solutions:—

Solution No. 1.

Copper sulphate recrystallized	69.30 grms.
Distilled water to 500 c.c.	

Solution No. 2.

Tartrated soda crystallized	175.00 grms.
Caustic soda (pure)	76.56 "
Distilled water to 500 c.c.	

Solution No. 3.

Cyanide of potassium (98 per cent)	33.00 grms.
Distilled water to 500 c.c.	

For the purpose of testing the solutions, 5 c.c. of each are mixed with 50 c.c. of distilled water, then boiled. Whilst boiling add a solution of grape sugar until the blue colour is discharged. If any precipitate is formed, more cyanide must be added to No. 3 until again on boiling equal volumes of the mixed solutions with grape sugar, they cease to precipitate.

As compared with Fehling's solution, the advantage is that the end reaction is very sharp, filtration is avoided, time is saved, and experimental error reduced.

The following are the details of a grape sugar estimation, when using what may be termed the cyano-cupric test. Measure 5 c.c. each of solutions No. 1, 2, and 3 in the order given; add 50 c.c. water, and boil in a porcelain capsule. Run the sugar solution into the boiling test solution until the blue colour has gone. This should be added slowly towards the end of the reaction. For accurate determination it is usual to make a second and more rapid estimation, so as to check error that may arise from too long boiling.

It is to be noted that the copper solution here recommended is twice the strength of Fehling's solution, but it is best to standardize the solution against a known weight of inverted cane sugar.

The Reduction of Mercury Salts.—Knapp recommends the use of an alkaline solution of potassio-mercuric cyanide. He prepares a standard solution by dissolving 10 grms. of pure mercuric cyanide in water, adding 100 c.c. of solution of sodium hydroxide of specific gravity 1.145, and making the solution up to 1000 c.c.; of this solution 10 c.c. are reduced by 25 milligrams of dextrose. The process of reduction is carried out as follows: 10 c.c. of the mercury solution and 25 c.c. of water are heated to boiling, and the sugar solution (of about $\frac{1}{2}$ per cent strength) is run in from a burette until the whole of the mercury is precipitated. To determine the end of the reaction, the precipitate is allowed to subside, and a drop of the supernatant liquid is spotted on to a piece of thin white filter paper. This paper is held for a few seconds over fuming hydrochloric acid and then exposed to sulphuretted hydrogen. If any mercury be left in solution a yellow or brown stain is at once produced on the spot.

Sachsse proposes the use of a solution containing 18 grms. of pure mercuric iodide, with 25 grms. of potassium iodide and 80 grms. of caustic potash in 1000 c.c. The titration is conducted with the sugar solution into the boiling mercury solution, the end of the reaction being determined when a drop of the supernatant liquid ceases to give a brown coloration with a drop of a strongly alkaline solution of stannous chloride. Ten c.c. of Sachsse's solution are reduced by 33 mgs. of dextrose or 27 mgs. of invert sugar.

According to various experimenters, the conditions under which the reduction of either copper or mercury salts is carried out, affect the results to a considerable extent, so that if accurate results are to be expected the titrations should be carried out against experiments with known quantities of sugar.

The *relative* reducing powers of the following sugars, taking that of dextrose as 100 for each solution, are as follows:—

	Fehling's.	Knapp's.	Sachs's.
Dextrose	100	100	100
Invert sugar	100	100	120 (?)
Levulose	100	100	148 (?)
Lactose	74	70	71
Maltose	62	64	65

For numerous modifications of Fehling's process, none of which appear to possess any particular advantage over the original method, references may be made to ("Journ. Amer. Chem. Soc." 1896, 749), ("Zeit. Anal. Chemie." 12, 27), ("Journ. Amer. Chem. Soc." 1907, 1744) and ("Zeit. Ver. Deut. Zuckerind." 1906, 1012).

The Polarimetric Determination of the Sugars.—Sugars, in general, possess the power of rotating the plane of plane-polarized light. The observation of this power of rotation is not conveniently effected on the solid sugar, but is determined on a solution of the solid substance. The rotation effected is approximately proportional to the concentration of the solution, but not strictly so. In determining this value, it must be remembered that certain sugars possess the power of bi-rotation, and their solutions should be allowed to stand for several hours before a reading is taken.

The bi-rotation of sugars may be destroyed by adding a few drops of strong ammonia to the solution before making it up to its normal volume, or by boiling the solution for a few minutes. The state of optical equilibrium is thus at once produced.

The specific rotatory power of an optically active substance is the angular rotation effected on the plane of polarization by causing it to traverse a thickness of 1 decimetre of the substance.¹ This power is different for different parts of the spectrum; hence it is usual to indicate the particular light which has been polarized. The symbol used for specific rotatory power is $[\alpha]$: that for the sodium light, or D line of the spectrum, which is the usual light used, being indicated by $[\alpha]_d$.

In practice it is usual to determine the angle of rotation for a solution of known concentration, and from this to calculate the specific rotatory power. Since, as has been mentioned above, the concentration is not always accurately in proportion to the observed angle, exact results are only obtaining by always using solutions of approximately constant concentration. The specific rotation of a substance in solution is calculated from the following formula:—

$$[\alpha] = \frac{100 \alpha}{lc}$$

¹ A more scientific, and stricter, definition of specific rotatory power is one which takes into account the density of the liquid.

The specific rotatory power of a liquid is the angle through which the plane of polarized light is turned, when the light traverses a layer whose thickness is inversely proportional to the specific gravity of the liquid. The decimetre is usually adopted as the unit of length.

Where $[\alpha]$ is the specific rotation, α the observed angle of rotation of the solution, l the length of the tube in decimetres, and c the number of grms. of substance in 100 c.c. of the solution. So long as one is dealing, then, with a solution of only one sugar, it is obvious that the percentage present in a solution can be calculated so long as the specific rotation of the sugar be known. The following are the mean values of numerous determinations of the apparent specific rotations of the more common sugars, for solutions containing about 10 per cent and for solutions containing about 16 per cent of the sugar. The values are for sodium light $[\alpha]_d$, and for the transition tint as used on some instruments, $[\alpha]_j$, at 15° C.

Sugar.	$[\alpha]_d$.		$[\alpha]_j$.	
	10 per cent	16 per cent	10 per cent	16 per cent
Cane sugar	+ 66.6°	+ 66.5°	+ 73.8	+ 73.6°
Maltose (anhydrous)	+ 138°	+ 138°	+ 154.5°	+ 154.3°
" (hydrated)	+ 132.2°	+ 139.4°	—	—
Lactose + 1H ₂ O	+ 52°	+ 52°	+ 58.5°	—
Dextrose	+ 53°	—	+ 58.5°	+ 58.3°
Levulose	— 98.8°	— 98.4°	— 109.7°	— 109.5°
Invert sugar	— 23.7°	— 28.6°	— 25.6°	—

(The rotation of levulose, and, consequently, of invert sugar, is affected greatly by temperature—see below.)

Brown and Millar ("Trans. Chem. Soc." 1897, 71, 73) give the following table for converting $[\alpha]_d$ into $[\alpha]_j$:—

Sugar.	Per cent solution.	$[\alpha]_j = [\alpha]_d \times by.$
Cane sugar	10	1.107
Maltose	10	1.113
"	5	1.111
Dextrose	10	1.115
"	5	1.111
Starch sugar	10	1.111
"	5	1.111

In practice, certain polarimeters are graduated on a scale which indicates the percentage of sugar present in a given solution, under definite conditions, whilst others are graduated in angular degrees as well. So far as angular degrees are concerned, the above formula

$$[\alpha] = \frac{100 \alpha}{lc} \text{ will always apply when only one active sugar is present.}$$

The graduations to read off the percentage of sugar present in the solution are based on the use of an amount of sucrose in 100 c.c. which will in a 2 decimetre tube cause the same rotation as a plate of quartz 1 millimetre in thickness. This, for any given instrument,

is known as the "normal weight". For other sugars than sucrose, angular rotations in degrees should be observed.

In the Soleil-Dubosq instrument 16.350 grms. of sucrose are taken as the normal weight in 100 c.c. of liquid. For other instruments of this type, quantities varying from 16.19 to 16.35 have been adopted—so that the value may be agreed as 16.35 grms. For polarimeters of the Ventzke type the standard weight, 26.048 grms., should be used (or 26 grms. if the strict metric c.c. be employed). If angular determinations are to be made solutions of 16 per cent to 18 per cent strength should be employed.

The amount of sugar used for graduating each particular type of instrument is indicated by the maker, and should be adhered to in making determinations. The standard instruments are graduated so that, for the transition tint 24 angular degrees (the rotation produced by 1 mg. of quartz or the standard weight of sugar in 100 c.c., in a 2 decimetre tube), or for the sodium light about 21.8° (the similar value), are divided into 100 sugar degrees. In making an observation, the standard weight of the sample is dissolved in water to 100 c.c. of solution and the reading taken, when the percentage of pure sucrose will be directly indicated on the scale. If calculations are to be made on instruments graduated in angular degrees, the percentage is determined by comparing the rotation of a solution with that of a solution of pure sucrose of the same concentration. Thus if a solution of 20 grms. of the sample in 100 c.c. give a rotation of $+25^\circ$ in a 2 decimetre tube, whilst an equally concentrated solution of pure sucrose gives a rotation of $+26.6^\circ$, the percentage of true sucrose in the sample is $\frac{25 \times 100}{26.6} = 94.3$ per cent.

As 18.8 grms. of sucrose in 100 c.c. in a 2 decimetre tube effect an angular rotation of exactly 25° , it follows that if exactly this weight of the sample be used, each angle of rotation equals 4 per cent of sugar in the sample, thus facilitating calculation. In determining the amount of sucrose in liquids of unknown strength, it is obvious that where an instrument graduated in angular degrees is used, the formula

$$[\alpha] = \frac{100 \ a}{lc}$$

applies, $[\alpha]$ being either 66.5° for sodium light, or 73.6° for white light with the neutral tint.

If the polarimeter be graduated in percentages of sugar, the concentration of the liquid is given by multiplying the standard weight of sugar for which the instrument is designed by the observed number of sugar degrees and dividing by 100.

The table at top of opposite page comparing the various instruments will be found useful.

Very frequently solutions of sugar are not in a fit state for the polarimeter, as no reading can be obtained unless the solution be both clear and very pale in colour. If the solution be dark coloured it should be agitated with about 20 per cent of its weight of fresh, dry bone black, and agitated from time to time and then filtered, or, as

German Instruments, such as	Normal Weight of Sugar.	1 Sugar Division = Angular Degrees (for Sodium Light).	1 Angular Degree = Sugar Divisions.
Schmidt, and Haensch, Ventzke, Scheibler, etc.	26.048	0.3468	2.8835
Soleil-Dubosq	16.35	0.2175	4.597
Laurent	16.27	0.2167	4.6154

preferred by A. H. Allen, the following method may be adopted. The normal quantity of sugar sample is weighed out and dissolved in about 50 c.c. of water in 100 c.c. flask. The solution may be (1) colourless, but cloudy, (2) yellow, (3) brown, (4) black. In the first case add about 3 c.c. of a cream of hydrated alumina (prepared by precipitating a solution of alum by a hot solution of sodium carbonate and washing the precipitate with hot water and then mixing it with enough water to form a thin cream) and one drop of a 40 per cent solution of basic acetate of lead. In the second case, the same amount of alumina should be added, but 3 to 5 drops of the lead solution. In the third and fourth cases, about 2 c.c. of a 10 per cent solution of sodium sulphite should be added, and then the lead solution gradually until no further precipitation takes place. The liquid is well agitated, the precipitate allowed to settle, and then made up to the 100 c.c. mark with water (the froth may be destroyed by the cautious addition of a drop or two of methylated spirit or ether). The liquid is now filtered and the rotation observed.

Bryan ("International Sugar Journal," 1908, 602) has shown, however, that basic lead acetate causes a precipitation of both dextrose and levulose, whereas normal lead acetate causes practically no such precipitation. The American Association of Official Agricultural Chemists now use only the normal acetate for the purpose of clarification. Eynon has shown that so long as only sufficient lead acetate is used to leave only a very slight excess in solution, no serious error results by the use of the basic salt, but considerable excess of lead causes (in Clerget's process) an increase in the direct polarization, and a decrease in the inversion polarization. A given sample for example gave the following results:—

c.c. of Basic Lead Acetate Solution (21 per cent. Pt.).	Direct Polarization.	Sucrose per cent (apparent).	Reducing Sugar per cent (apparent).
6	73.2°	76	9.7
8	73.4°	76.1	9.6
26	74°	76.3	9.1
50	75.1°	76.5	8.5

Pellet ("Bull. Soc. Chem. Sucr. et Dist." 1906, **23**, 1466) states that the error due to clarifying sugar solutions with basic acetate of lead, due to the volume occupied by the precipitate, is compensated by the small amount of sugar mechanically precipitated with the lead compounds, and that no correction is necessary.

In dealing with sugars other than sucrose (so long as the standard weight of the sample is used) the observed percentage recorded on the instrument which is graduated in sugar units may be corrected with actual percentages of the sugar in question by multiplying by the factor $\frac{[\alpha]^1}{[\alpha]^2}$ where $[\alpha]^1$ is the specific rotation of cane sugar, and $[\alpha]^2$ is that of the sugar in question. If angular degrees be employed the formula $[\alpha] = \frac{100a}{lc}$ stands good, where $[\alpha]$ is the specific rotation, a the observed angle, l the length of the tube in decimetres and c the number of grammes per 100 c.c. of the actual sugar.

As a rule polarimetric observations are valueless when more than one optically active substance is present. In certain cases, however, the presence of two such bodies does not prevent a fairly accurate determination being made. This is the case where one of the bodies in question does not alter its optical properties by certain treatment, whilst the other one does, and this alteration is capable of determination. For example, Clerget has proposed the hydrolysis of cane sugar in the presence of glucose (dextrose) and the determination of the optical properties of the inverted sugars in comparison with the same values before inversion, as a means of determining the amount of sucrose present when mixed with dextrose.

Dextrose is not affected by heating with dilute acids, whilst sucrose is converted into "invert" sugar—a mixture in equal quantities of dextrose and levulose, 100 parts of these being yielded by 95 parts of sugar. The specific rotations of both sucrose and dextrose are not materially affected by change in temperature, whereas that of levulose is markedly affected. From a specific rotation of about -95° at 20° , this value falls to -53° at 87.2° .

As a matter of experiment it has been found that a solution of sucrose which causes a rotation of 100 sugar degrees to the right in a 2 decimetre tube, will have a rotation of 39 degrees to the left after inversion, the reading being taken at $10^\circ C.$, and has therefore undergone a change of 139 divisions. The difference is less, the higher the temperature, a reduction of 1 degree for each 2° of temperature taking place. Hence at 0° , the difference in 144 sugar degrees, and, of course, for any other temperature is given by the equation

$$D = 144 - \frac{t}{2}, \text{ where } t \text{ is the temperature centigrade.}$$

Care should be taken that either the bulk of the solution is identical before and after inversion, or if it be increased after inversion it should be by 10 per cent, and the readings taken before inversion in a 200 mm. tube, and after inversion in a 220 mm. tube, when no correction will be necessary.

The readings before and after inversion must be taken at the same temperature, 15° being most suitable. At this temperature the change by inversion is 136.5° , so that the observed change in rotation, multiplied by $\frac{100}{136.5}$ (or 0.7326) represents the rotation due to the original sucrose in the solution, from which the amount of cane sugar may be deduced. This factor stands good for 15° C. however the change in rotatory power be expressed, whether in sugar degrees, or in angular degrees.

Thus if a solution gives a rotatory power of $+20^{\circ}$ before inversion, and after inversion a rotation of -5° , then the actual change in rotatory power is 25° , which, multiplied by 0.7326, is 18.31° . Therefore the rotation of 18.31° is due to cane sugar originally present in the solution, and that of $+1.69^{\circ}$ to dextrose (or some other dextro-rotatory substance).

Clerget's original formula, then, is

$$S = \frac{100K}{144 - 0.5t^{\circ}}$$

where S equals the rotation in the original solution due to sucrose, K equals the observed difference in rotation before and after inversion, and t° = the temperature centigrade.

The polarimeter employed by Clerget was a Soleil instrument using 16.471 grms. of sugar as its standard. The usual polarimeter employed by sugar analysts in this country is a Soleil-Ventzke-Scheibler, or some modification of it, using 26 grms., and with this instrument the figures of Clerget are not strictly accurate, although very nearly so.

Herzfeld has investigated the inversion values more recently and his figures are now accepted universally as accurate. Using the last-named standard amount of sugar—viz. 13 grms. per 100 c.c., he finds 132.66° as the difference figure before and after inversion, if the readings be taken at 20° , which is equal to 142.66° at 0° . Hence the formula of Clerget, corrected for present instruments becomes

$$S = \frac{100K}{142.66 - 0.5t^{\circ}}$$

If any other concentration be employed, the inversion constant varies slightly: the following are the values for given concentration, at 0° C. :—

Per cent
1 = 141.85
5 = 142.12
10 = 142.46
15 = 142.79
20 = 143.13

Inversion is usually best carried out by heating the solution with 10 per cent of its volume of strong hydrochloric acid at 68° to 70° (the time taken to attain this temperature should be about ten minutes) and then cooling down by immersion in cold water. If 50 c.c. be thus

made up to 55 c.c., the reading in a 220 mm. tube will be comparable with the reading of the original solution in a 200 mm. tube.

It is to be noted that heating for ten minutes at about 70° with 10 per cent of hydrochloric acid completely inverts sucrose, but has little action on maltose. To invert maltose, heating with 3 per cent of strong sulphuric acid at 100° for three to four hours is advisable.

Hence an approximation to the amounts of sucrose and maltose in a mixture can be obtained by using these two methods of inversion.

Pierrraerts ("Bull. Assoc. Chim. Sucr. et Dist." 1909, 650) gives the following formula for the determination of mixtures of sucrose and maltose, when examined by the polarimeter before and after inversion. Taking 66.5 as the specific rotation of sucrose and 130 as that of hydrated maltose, and denoting the quantity of sucrose per 100 c.c. of the hydrolysed solution by x , and the corresponding quantity of maltose by y , then

$$\begin{aligned}x &= 0.57246 (a - a') \\y &= 0.3846154a - 0.2928363 (a - a')\end{aligned}$$

where a is the polarimetric reading in sugar degrees before inversion and a' is the reading after inversion.

Mixtures of Sucrose, Invert Sugar and Glucose.—Boseley ("Analyst," xxii. 123) has published a number of observations on the analysis of marmalade, which may be taken also to apply generally to the examination of jams.

No difficulties are presented in the determination of the water or free acids, the only point of real importance being an examination of the sugars present. Supposing only cane sugar and invert sugar to be present (due to the action of the acids of the fruit on the sucrose used), the following method is the best to employ:—

65.12 grms. of the well-mixed sample are weighed out, and mixed with about 50 c.c. of cold water: this is decanted into a 250 c.c. flask and successive quantities of about 50 c.c. of water are used until the whole is transferred to the flask. Add solution of basic acetate of lead, make up to 250 c.c. and shake well. Excess of lead acetate should be avoided, by seeing that the solution remains slightly acid. The liquid is filtered and the polarimetric value taken. Fifty c.c. of the filtrate are then treated with 5 c.c. of strong HCl, and inverted in the usual manner. The cane sugar is calculated from the difference in the polarization by Clerget's formula (see p. 132) and the invert sugar from the formula

$$\text{Invert sugar} = \frac{(\text{cane sugar} - \text{direct reading})100}{44 - \frac{t}{2}}.$$

If glucose be present it will be indicated by the reading after inversion being positive instead of negative, or at all events much smaller than usual if it be negative. If this be the case it will be necessary to determine the cupric reducing power.

This is best done, on the assumption that the reducing power of marmalade is in the neighbourhood of 25 per cent of sugar, by pre-

paring a solution of 13.024 grms. of pure cane sugar in 100 c.c. and inverting by acid in the usual manner. Make up to 110 c.c. and take 11 c.c. and dilute to 100 c.c. Call this solution A.

Now take 20 c.c. of the filtrate of the marmalade solution (65.12 grms. in 250 c.c.) and dilute to 100 c.c. Call this solution B. It contains four times as much of the marmalade, as solution A does sugar. Boil in the usual manner with alkaline copper tartrate solution, using 10 c.c. of each solution in two small beakers, with the usual precautions, when the cupric reducing power of the sample, calculated into percentage of invert sugar, will be given by the following:—

$$\frac{\text{Cu obtained from B}}{\text{four times Cu from A}} \times 100.$$

The approximate amounts of cane sugar, invert sugar and glucose in a marmalade can be calculated from the following:—

$$\text{Cane sugar} = \frac{100 \times (\text{direct} - \text{inverted reading})}{144 - \frac{t}{2}}$$

$$\text{Invert sugar} = \frac{\text{Cane sugar} - \text{direct reading} + (4 \times (\text{cupric reducing power}))}{4 + \frac{44 - \frac{t}{2}}{100}}$$

$$\text{Glucose} = 2(\text{cupric reducing power} - \text{invert sugar})$$

NOTES.—

The cupric reducing power is in terms of invert sugar.

Glucose is assumed to contain 81.9 per cent of solids.

The factor 144 in Clerget's formula should be 142.66; and consequently 44 in the same formula should be 42.66.

COMMERCIAL CANE SUGAR AND ITS PRODUCTS.

Commercial sugar is manufactured either from the sugar cane, or from the sugar beetroot, the latter forming the source of supply of the bulk of the sugar manufactured on the European continent. Beetroot sugar is the variety usually speculated in on the London market, a polarization test being the accepted basis of sugar contracts. The amount of cane sugar obtained from other sources is insignificant.

The principal types of sugar one meets with are as follows;—

- (1) Pure sugar, in the form of cones (loaves), cubes, small crystals or large crystals (sugar candy), and powder.
- (2) Brown crystals, often containing 97 per cent of sucrose.
- (3) Raw sugars, containing about 88 per cent of true sucrose.
- (4) Molasses or treacle. This is the syrup which is left after the crystallization of the sucrose, and contains a considerable amount of sucrose with more or less glucose, etc.

The following analyses illustrate the average composition of various types of sucrose :—

Sugar.	Sucrose.	Glucose.	Ash.	Water.	Organic Matter not Sugar.	Authority.
Raw cane, W.I.	94.4	2.2	0.2	2.8	0.3	Wallace.
" "	88.0	5.14	0.96	4.23	1.67	Wigner and Harland
" "	90.4	5.47	0.36	4.22	1.55	" "
Raw beet (25 samples)	87 to 93.5	0 to 0.2	1.4 to 2.1	2 to 5.1	1.2 to 3.0	Parry
Pure cane (in various forms)	99.6 to 99.9	—	traces	traces	—	"
Pure beet loaves	99.1	trace	0.15	0.25	—	Wigner and Harland

A. H. Bryan ("U. S. Dept. Agriculture Bur. of Chem., Circular" No. 40) gives the following analyses of pure maple products :—

	Sugar.	Juice.
Water . . .	3.05 to 11 per cent	up to 32 per cent.
Sucrose . . .	72.6 ,, 87.4 ,,	51 ,, 62.2 ,,
Invert sugar . . .	1.16 ,, 8.37 ,,	0.34 ,, 9.17 ,,
Lead No . . .	1.83 ,, 2.48	1.19 ,, 2.03
Ash . . .	0.64 ,, 1.32 per cent	0.46 ,, 1.01 per cent
Soluble ash . . .	0.33 ,, 0.67 ,,	0.21 ,, 0.63 ,,

The only determinations necessary as a rule in examining commercial sugar, are the water, mineral matter, sucrose, reducing sugars—and the difference figure of these, which is usually returned as organic matter other than sugar.

The water is estimated by heating 5 grms. in a platinum capsule at 100° to 110° until the weight is constant. If, however, much glucose is present, the temperature should be kept below 65° C. and the time of drying consequently lengthened, since glucose increases in weight by prolonged exposure to heat.

Ash Determination.—The actual amount of ash of commercial sugar is difficult and tedious to ascertain exactly, since it is difficult to completely incinerate the sugar, and the ash is both very light and liable to be blown away, and hygroscopic and difficult to weigh. Its minute proportion, however, makes it a matter of almost indifference, if it be sulphated and weighed as sulphates. Some prefer to deduct 10 per cent of the weight of the sulphated ash to convert into true ash, but this refinement is scarcely necessary, considering its minute amount. It is usually returned in this method however. Three grms. of the sample are slightly moistened with water and then with a little pure, strong, sulphuric acid, and the whole gently heated to a cinder, when it is burned at a low red heat in a muffle, being moistened again with sulphuric acid when it is nearly free from carbon. The presence

of sand or clay will be indicated by a high ash value, and by a high proportion of matter insoluble in acid. The average value of the ash in pure refined sugar is from a mere trace to 0.1 per cent, whilst in raw sugar it may reach 2 or even 3 per cent.

Monier gives the following as the average composition of the ash of cane and beet sugars :—

	Cane Sugar.	Beet Sugar.
Alkaline carbonates	16.5	82.2
Calcium carbonates	49.0	6.7
Alkaline sulphates	16.0	} 11.1
Sodium chloride	9.0	
SiO ₂ and Al ₂ O ₃	9.5	
		none

Actual Sugar.—Sucrose may be estimated by a direct polarimetric reading on the principles given above. If no glucose, or practically none is present, a direct reading is sufficiently accurate, but in the presence of reducing sugars, Clerget's inversion process, as described on page 132, should be used. If necessary, the sugar may be estimated by inversion and the reduction of Fehling's solution, but the results are not so accurate as the more simple polarimetric method.

Invert Sugar may be estimated by any of the copper or mercury reduction processes described above. If the solutions are dark coloured, the polarimetric process is preferable—and probably more accurate. Any dextrose which may have been added intentionally will be determined by either process.

It is usually sufficient to return the difference figures as "organic matter other than sugar". But for the purposes of the sugar refiner, it is sometimes necessary to decide whether much gummy or albuminous matter be present, as such bodies have a deleterious effect on crystallization.

The Refractive Index of Sugar Solutions.—If, as is often the case, the estimation of cane sugar in a solution containing nothing else than that sugar, be required, the determination of the refractive index will yield the required information.

The following table, showing the amount of water in syrups as indicated by their refractive indices, is due to Main :—

Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.
1.3330	100	1.3406	94.6	1.3488	89.2	1.3574	83.8
1.3331	99.9	1.3408	94.5	1.3489	89.1	1.3576	83.7
1.3333	99.8	1.3409	94.4	1.3491	89	1.3577	83.6
1.3334	99.7	1.3411	94.3	1.3492	88.9	1.3579	83.5
1.3336	99.6	1.3412	94.2	1.3494	88.8	1.3581	83.4
1.3337	99.5	1.3414	94.1	1.3496	88.7	1.3582	83.3
1.3338	99.4	1.3415	94	1.3497	88.6	1.3584	83.2
1.3340	99.3	1.3417	93.9	1.3499	88.5	1.3586	83.1
1.3341	99.2	1.3418	93.8	1.3500	88.4	1.3587	83
1.3343	99.1	1.3420	93.7	1.3502	88.3	1.3589	82.9
1.3344	99	1.3421	93.6	1.3503	88.2	1.3591	82.8
1.3345	98.9	1.3423	93.5	1.3505	88.1	1.3592	82.7
1.3347	98.8	1.3424	93.4	1.3507	88	1.3594	82.6
1.3348	98.7	1.3426	93.3	1.3508	87.9	1.3596	82.5
1.3350	98.6	1.3427	93.2	1.3510	87.8	1.3597	82.4
1.3351	98.5	1.3429	93.1	1.3511	87.7	1.3599	82.3
1.3352	98.4	1.3430	93	1.3513	87.6	1.3600	82.2
1.3354	98.3	1.3432	92.9	1.3515	87.5	1.3602	82.1
1.3355	98.2	1.3433	92.8	1.3516	87.4	1.3604	82
1.3357	98.1	1.3435	92.7	1.3518	87.3	1.3605	81.9
1.3358	98	1.3436	92.6	1.3519	87.2	1.3607	81.8
1.3359	97.9	1.3438	92.5	1.3521	87.1	1.3609	81.7
1.3361	97.8	1.3439	92.4	1.3522	87	1.3610	81.6
1.3362	97.7	1.3441	92.3	1.3524	86.9	1.3612	81.5
1.3364	97.6	1.3442	92.2	1.3526	86.8	1.3614	81.4
1.3365	97.5	1.3444	92.1	1.3527	86.7	1.3615	81.3
1.3366	97.4	1.3445	92	1.3529	86.6	1.3617	81.2
1.3368	97.3	1.3447	91.9	1.3530	86.5	1.3619	81.1
1.3369	97.2	1.3448	91.8	1.3532	86.4	1.3620	81
1.3371	97.1	1.3450	91.7	1.3533	86.3	1.3622	80.9
1.3372	97	1.3451	91.6	1.3535	86.2	1.3624	80.8
1.3373	96.9	1.3453	91.5	1.3537	86.1	1.3625	80.7
1.3375	96.8	1.3454	91.4	1.3538	86	1.3627	80.6
1.3376	96.7	1.3456	91.3	1.3540	85.9	1.3629	80.5
1.3378	96.6	1.3457	91.2	1.3541	85.8	1.3630	80.4
1.3379	96.5	1.3459	91.1	1.3543	85.7	1.3632	80.3
1.3380	96.4	1.3460	91	1.3545	85.6	1.3634	80.2
1.3382	96.3	1.3462	90.9	1.3546	85.5	1.3635	80.1
1.3383	96.2	1.3463	90.8	1.3548	85.4	1.3637	80
1.3385	96.1	1.3465	90.7	1.3549	85.3	1.3639	79.9
1.3386	96	1.3466	90.6	1.3551	85.2	1.3640	79.8
1.3387	95.9	1.3468	90.5	1.3552	85.1	1.3642	79.7
1.3389	95.8	1.3469	90.4	1.3554	85	1.3644	79.6
1.3390	95.7	1.3471	90.3	1.3556	84.9	1.3645	79.5
1.3392	95.6	1.3472	90.2	1.3557	84.8	1.3647	79.4
1.3393	95.5	1.3474	90.1	1.3559	84.7	1.3649	79.3
1.3394	95.4	1.3475	90	1.3561	84.6	1.3650	79.2
1.3396	95.3	1.3477	89.9	1.3562	84.5	1.3652	79.1
1.3397	95.2	1.3478	89.8	1.3564	84.4	1.3654	79
1.3399	95.1	1.3480	89.7	1.3566	84.3	1.3655	78.9
1.3400	95	1.3481	89.6	1.3567	84.2	1.3657	78.8
1.3402	94.9	1.3483	89.5	1.3569	84.1	1.3659	78.7
1.3403	94.8	1.3484	89.4	1.3571	84	1.3661	78.6
1.3405	94.7	1.3486	89.3	1.3572	83.9	1.3662	78.5

Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.
1-3664	78.4	1-3757	73	1-3854	67.6	1-3955	62.2
1-3666	78.3	2-3758	72.9	1-3856	67.5	1-3957	62.1
1-3667	78.2	1-3760	72.8	1-3858	67.4	1-3959	62
1-3669	78.1	1-3762	72.7	1-3860	67.3	1-3961	61.9
1-3671	78	1-3764	72.6	1-3862	67.2	1-3963	61.8
1-3672	77.9	1-3766	72.5	1-3863	67.1	1-3965	61.7
1-3674	77.8	1-3767	72.4	1-3865	67	1-3967	61.6
1-3676	77.7	1-3769	72.3	1-3867	66.9	1-3969	61.5
1-3677	77.6	1-3771	72.2	1-3869	66.8	1-3970	61.4
1-3679	77.5	1-3773	72.1	1-3871	66.7	1-3972	61.3
1-3681	77.4	1-3774	72	1-3873	66.6	1-3974	61.2
1-3682	77.3	1-3776	71.9	1-3874	66.5	1-3976	61.1
1-3684	77.2	1-3778	71.8	1-3876	66.4	1-3978	61
1-3686	77.1	1-3780	71.7	1-3878	66.3	1-3980	60.9
1-3687	77	1-3782	71.6	1-3880	66.2	1-3982	60.8
1-3689	76.9	1-3783	71.5	1-3882	66.1	1-3984	60.7
1-3691	76.8	1-3785	71.4	1-3884	66	1-3986	60.6
1-3692	76.7	1-3787	71.3	1-3885	65.9	1-3988	60.5
1-3694	76.6	1-3789	71.2	1-3887	65.8	1-3989	60.4
1-3696	76.5	1-3790	71.1	1-3889	65.7	1-3991	60.3
1-3697	76.4	1-3792	71	1-3891	65.6	1-3993	60.2
1-3699	76.3	1-3794	70.9	1-3893	65.5	1-3995	60.1
1-3701	76.2	1-3796	70.8	1-3895	65.4	1-3997	60
1-3703	76.1	1-3798	70.7	1-3896	65.3	1-3999	59.9
1-3704	76	1-3799	70.6	1-3898	65.2	1-4001	59.8
1-3706	75.9	1-3801	70.5	1-3900	65.1	1-4003	59.7
1-3708	75.8	1-3803	70.4	1-3902	65	1-4005	59.6
1-3709	75.7	1-3805	70.3	1-3904	64.9	1-4007	59.5
1-3711	75.6	1-3806	70.2	1-3906	64.8	1-4009	59.4
1-3713	75.5	1-3808	70.1	1-3908	64.7	1-4011	59.3
1-3714	75.4	1-3810	70	1-3910	64.6	1-4013	59.2
1-3716	75.3	1-3812	69.9	1-3912	64.5	1-4015	59.1
1-3718	75.2	1-3814	69.8	1-3913	64.4	1-4017	59
1-3719	75.1	1-3816	69.7	1-3915	64.3	1-4019	58.9
1-3721	75	1-3817	69.6	1-3917	64.2	1-4021	58.8
1-3723	74.9	1-3819	69.5	1-3919	64.1	1-4022	58.7
1-3725	74.8	1-3821	69.4	1-3921	64	1-4024	58.6
1-3726	74.7	1-3823	69.3	1-3923	63.9	1-4026	58.5
1-3728	74.6	1-3825	69.2	1-3925	63.8	1-4028	58.4
1-3730	74.5	1-3827	69.1	1-3927	63.7	1-4030	58.3
1-3732	74.4	1-3828	69	1-3929	63.6	1-4032	58.2
1-3733	74.3	1-3830	68.9	1-3931	63.5	1-4034	58.1
1-3735	74.2	1-3832	68.8	1-3932	63.4	1-4036	58
1-3737	74.1	1-3834	68.7	1-3934	63.3	1-4038	57.9
1-3739	74	1-3836	68.6	1-3936	63.2	1-4040	57.8
1-3541	73.9	1-3838	68.5	1-3938	63.1	1-4042	57.7
1-3742	73.8	1-3839	68.4	1-3940	63	1-4044	57.6
1-3744	73.7	1-3841	68.3	1-3942	62.9	1-4046	57.5
1-3746	73.6	1-3843	68.2	1-3944	62.8	1-4048	57.4
1-3748	73.5	1-3845	68.1	1-3946	62.7	1-4050	57.3
1-3749	73.4	1-3847	68	1-3948	62.6	1-4052	57.2
1-3751	73.3	1-3849	67.9	1-3950	62.5	1-4054	57.1
1-3753	73.2	1-3850	67.8	1-3951	62.4	1-4056	57
1-3755	73.1	1-3852	67.7	1-3953	62.3	1-4058	56.9

Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.
1.4060	56.8	1.4171	51.4	1.4283	46	1.4405	40.6
1.4062	56.7	1.4173	51.3	1.4285	45.9	1.4408	40.5
1.4064	56.6	1.4176	51.2	1.4288	45.8	1.4410	40.4
1.4066	56.5	1.4178	51.1	1.4290	45.7	1.4412	40.3
1.4068	56.4	1.4180	51	1.4292	45.6	1.4414	40.2
1.4070	56.3	1.4182	50.9	1.4294	45.5	1.4417	40.1
1.4071	56.2	1.4184	50.8	1.4296	45.4	1.4419	40
1.4073	56.1	1.4186	50.7	1.4298	45.3	1.4421	39.9
1.4075	56	1.4188	50.6	1.4300	45.2	1.4424	39.8
1.4077	55.9	1.4190	50.5	1.4302	45.1	1.4426	39.7
1.4079	55.8	1.4193	50.4	1.4304	45	1.4428	39.6
1.4081	55.7	1.4195	50.3	1.4306	44.9	1.4431	39.5
1.4083	55.6	1.4197	50.2	1.4309	44.8	1.4433	39.4
1.4085	55.5	1.4199	50.1	1.4311	44.7	1.4435	39.3
1.4087	55.4	1.4201	50	1.4313	44.6	1.4438	39.2
1.4089	55.3	1.4203	49.9	1.4316	44.5	1.4440	39.1
1.4091	55.2	1.4205	49.8	1.4318	44.4	1.4442	39
1.4093	55.1	1.4207	49.7	1.4320	44.3	1.4445	38.9
1.4095	55	1.4209	49.6	1.4322	44.2	1.4447	38.8
1.4097	54.9	1.4211	49.5	1.4325	44.1	1.4449	38.7
1.4099	54.8	1.4213	49.4	1.4327	44	1.4451	38.6
1.4101	54.7	1.4215	49.3	1.4329	43.9	1.4454	38.5
1.4103	54.6	1.4217	49.2	1.4332	43.8	1.4456	38.4
1.4106	54.5	1.4220	49.1	1.4334	43.7	1.4458	38.3
1.4108	54.4	1.4222	49	1.4336	43.6	1.4461	38.2
1.4110	54.3	1.4224	48.9	1.4339	43.5	1.4463	38.1
1.4112	54.2	1.4226	48.8	1.4341	43.4	1.4465	38
1.4114	54.1	1.4228	48.7	1.4343	43.3	1.4468	37.9
1.4116	54	1.4230	48.6	1.4345	43.2	1.4470	37.8
1.4118	53.9	1.4232	48.5	1.4348	43.1	1.4472	37.7
1.4120	53.8	1.4234	48.4	1.4350	43	1.4475	37.6
1.4123	53.7	1.4236	48.3	1.4352	42.9	1.4477	37.5
1.4125	53.6	1.4238	48.2	1.4355	42.8	1.4479	37.4
1.4127	53.5	1.4240	48.1	1.4357	42.7	1.4482	37.3
1.4129	53.4	1.4242	48	1.4359	42.6	1.4484	37.2
1.4131	53.3	1.4244	47.9	1.4362	42.5	1.4486	37.1
1.4133	53.2	1.4246	47.8	1.4364	42.4	1.4489	37
1.4135	53.1	1.4248	47.7	1.4366	42.3	1.4491	36.9
1.4137	53	1.4250	47.6	1.4368	42.2	1.4493	36.8
1.4140	52.9	1.4253	47.5	1.4371	42.1	1.4496	36.7
1.4142	52.8	1.4255	47.4	1.4373	42	1.4498	36.6
1.4144	52.7	1.4257	47.3	1.4375	41.9	1.4500	36.5
1.4146	52.6	1.4259	47.2	1.4378	41.8	1.4503	36.4
1.4148	52.5	1.4261	47.1	1.4380	41.7	1.4505	36.3
1.4150	52.4	1.4263	47	1.4382	41.6	1.4507	36.2
1.4152	52.3	1.4265	46.9	1.4385	41.5	1.4509	36.1
1.4154	52.2	1.4267	46.8	1.4387	41.4	1.4512	36
1.4156	52.1	1.4269	46.7	1.4389	41.3	1.4514	35.9
1.4159	52	1.4271	46.6	1.4391	41.2	1.4516	35.8
1.4161	51.9	1.4273	46.5	1.4394	41.1	1.4519	35.7
1.4163	51.8	1.4275	46.4	1.4396	41	1.4521	35.6
1.4165	51.7	1.4277	46.3	1.4398	40.9	1.4523	35.5
1.4167	51.6	1.4279	46.2	1.4401	40.8	1.4526	35.4
1.4169	51.5	1.4281	46.1	1.4403	40.7	1.4528	35.3

Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.	Refractive Index at 20° C.	Water Per cent.
1.4530	35.2	1.4649	30.1	1.4774	25	1.4904	19.9
1.4533	35.1	1.4651	30	1.4777	24.9	1.4906	19.8
1.4535	35	1.4653	29.9	1.4779	24.8	1.4909	19.7
1.4537	34.9	1.4656	29.8	1.4782	24.7	1.4912	19.6
1.4540	34.8	1.4658	29.7	1.4784	24.6	1.4914	19.5
1.4542	34.7	1.4661	29.6	1.4787	24.5	1.4917	19.4
1.4544	34.6	1.4663	29.5	1.4789	24.4	1.4919	19.3
1.4547	34.5	1.4666	29.4	1.4792	24.3	1.4922	19.2
1.4549	34.4	1.4668	29.3	1.4794	24.2	1.4925	19.1
1.4551	34.3	1.4671	29.2	1.4797	24.1	1.4927	19
1.4554	34.2	1.4673	29.1	1.4799	24	1.4930	18.9
1.4556	34.1	1.4676	29	1.4802	23.9	1.4933	18.8
1.4558	34	1.4678	28.9	1.4804	23.8	1.4935	18.7
1.4561	33.9	1.4681	28.8	1.4807	23.7	1.4938	18.6
1.4563	33.8	1.4683	28.7	1.4810	23.6	1.4941	18.5
1.4565	33.7	1.4685	28.6	1.4812	23.5	1.4943	18.4
1.4567	33.6	1.4688	28.5	1.4815	23.4	1.4946	18.3
1.4570	33.5	1.4690	28.4	1.4817	23.3	1.4949	18.2
1.4572	33.4	1.4693	28.3	1.4820	23.2	1.4951	18.1
1.4574	33.3	1.4695	28.2	1.4822	23.1	1.4954	18
1.4577	33.2	1.4698	28.1	1.4825	23	1.4956	17.9
1.4579	33.1	1.4700	28	1.4827	22.9	1.4959	17.8
1.4581	33	1.4703	27.9	1.4830	22.8	1.4962	17.7
1.4584	32.9	1.4805	27.8	1.4832	22.7	1.4964	17.6
1.4586	32.8	1.4708	27.7	1.4835	22.6	1.4867	17.5
1.4588	32.7	1.4710	27.6	1.4838	22.5	1.4970	17.4
1.4591	32.6	1.4713	27.5	1.4840	22.4	1.4972	17.3
1.4593	32.5	1.4715	27.4	1.4843	22.3	1.4975	17.2
1.4595	32.4	1.4717	27.3	1.4845	22.2	1.4978	17.1
1.4598	32.3	1.4720	27.2	1.4848	22.1	1.4980	17
1.4600	32.2	1.4722	27.1	1.4850	22	1.4983	16.9
1.4602	32.1	1.4725	27	1.4853	21.9	1.4985	16.8
1.4605	32	1.4727	26.9	1.4855	21.8	1.4988	16.7
1.4607	31.9	1.4730	26.8	1.4858	21.7	1.4991	16.6
1.4609	31.8	1.4732	26.7	1.4860	21.6	1.4993	16.5
1.4612	31.7	1.4735	26.6	1.4863	21.5	1.4996	16.4
1.4614	31.6	1.4737	26.5	1.4865	21.4	1.4999	16.3
1.4616	31.5	1.4740	26.4	1.4868	21.3	1.5001	16.2
1.4619	31.4	1.4742	26.3	1.4871	21.2	1.5004	16.1
1.4621	31.3	1.4744	26.2	1.4873	21.1	1.5007	16
1.4623	31.2	1.4747	26.1	1.4876	21	1.5009	15.9
1.4625	31.1	1.4749	26	1.4878	20.9	1.5012	15.8
1.4628	31	1.4752	25.9	1.4881	20.8	1.5015	15.7
1.4630	30.9	1.4754	25.8	1.4883	20.7	1.5017	15.6
1.4632	30.8	1.4757	25.7	1.4886	20.6	1.5020	15.5
1.4635	30.7	1.4759	25.6	1.4888	20.5	1.5022	15.4
1.4637	30.6	1.4762	25.5	1.4891	20.4	1.5025	15.3
1.4639	30.5	1.4764	25.4	1.4893	20.3	1.5028	15.2
1.4642	30.4	1.4767	25.3	1.4896	20.2	1.5030	15.1
1.4644	30.3	1.4769	25.2	1.4898	20.1	1.5033	15
1.4646	30.2	1.4772	25.1	1.4901	20		

An approximate valuation of crude sugar may be made by making a saturated solution and taking the specific gravity of the solution at 17.5°. The percentage of sucrose and its impurities is indicated by the specific gravity and the following table gives the approximate values :—

Specific Gravity of Saturated Solution at 17.5°.	Percentage Composition of the Solution.		
	Sucrose.	Impurities.	Water.
1.330	66.66	—	33.34
1.3322	64.85	2.66	32.49
1.3384	63.70	5.29	31.01
1.3446	62.65	7.76	29.68
1.3509	61.42	10.13	28.45
1.3572	60.28	12.48	27.24
1.3636	59.14	14.67	26.19
1.3700	58.00	16.82	25.18
1.3764	57.85	18.87	24.28
1.3829	55.70	20.77	23.53
1.3894	54.56	22.59	22.85
1.3959	53.42	24.36	22.22
1.4025	52.28	25.98	21.74
1.4092	51.14	27.56	21.30
1.4159	50.00	29.00	21.00

The table on opposite page gives the specific gravity of pure sugar solutions (from 1 per cent to 74 per cent) at 17.5° C.

Adulteration of Sugar.—Coarse adulteration of sugar is not now common, and the old additions of sago and potato flour are now never met with. The improvement in this respect may be seen when one finds that prior to 1845 when the duty on sugar varied from 25s. to 63s. per cwt. according to the origin of the sugar, it has been estimated by competent authorities that from 10,000 to 12,000 tons of intentionally added matter was annually sold fraudulently as sugar!

To-day it is accidental impurities such as wood fibres and a small amount of clay or sandy matter that one meets with—and this principally in raw sugar.

Occasionally starch glucose in an anhydrous condition is met with. This is rarely added to refined sugar, but to moist sugar, sold as coffee sugar, which may contain, naturally, a little glucose.

But genuine sugar of this type will never give a copper reduction figure, corresponding to more than 4 per cent to 6 per cent of dextrose; hence any greater value obtained by Clerget's process will indicate the addition of glucose. If the starch glucose contain much dextrose and maltose, the initial rotation of the sugar may be such as to indicate more than 100 per cent of sucrose. Such a figure is definite evidence of adulteration.

Crystals of beet sugar are sometimes dyed in order to resemble the coloured Demerara crystals which are, of course, more valuable. The

TABLE SHOWING THE STRENGTH OF SUGAR SOLUTIONS BY SPECIFIC GRAVITY AT 17.5° C.

Sugar Per cent.	Specific Gravity according to		Sugar Per cent.	Specific Gravity according to	
	Balling.	Niemann.		Balling.	Niemann.
1	1.0040	1.0035	38	1.1692	1.1681
2	1.0080	1.0070	39	1.1743	1.1731
3	1.0120	1.0106	40	1.1794	1.1781
4	1.0160	1.0143	41	1.1846	1.1832
5	1.0200	1.0179	42	1.1898	1.1883
6	1.0240	1.0215	43	1.1951	1.1935
7	1.0281	1.0254	44	1.2004	1.1989
8	1.0322	1.0291	45	1.2057	1.2043
9	1.0363	1.0328	46	1.2111	1.2098
10	1.0404	1.0367	47	1.2165	1.2153
11	1.0446	1.0410	48	1.2219	1.2209
12	1.0488	1.0456	49	1.2274	1.2265
13	1.0530	1.0504	50	1.2329	1.2322
14	1.0572	1.0552	51	1.2385	1.2378
15	1.0614	1.0600	52	1.2441	1.2434
16	1.0657	1.0647	53	1.2499	1.2490
17	1.0700	1.0693	54	1.2553	1.2546
18	1.0744	1.0738	55	1.2610	1.2602
19	1.0788	1.0784	56	1.2667	1.2658
20	1.0832	1.0830	57	1.2725	1.2714
21	1.0877	1.0875	58	1.2783	1.2770
22	1.0922	1.0920	59	1.2841	1.2826
23	1.0967	1.0965	60	1.2900	1.2882
24	1.1013	1.1010	61	1.2959	1.2938
25	1.1059	1.1056	62	1.3019	1.2994
26	1.1106	1.1103	63	1.3079	1.3050
27	1.1153	1.1150	64	1.3139	1.3105
28	1.1200	1.1197	65	1.3190	1.3160
29	1.1247	1.1245	66	1.3260	1.3215
30	1.1295	1.1293	67	1.3321	1.3270
31	1.1343	1.1340	68	1.3383	1.3324
32	1.1391	1.1388	69	1.3445	1.3377
33	1.1440	1.1436	70	1.3507	1.3430
34	1.1490	1.1484	71	1.3570	1.3483
35	1.1540	1.1533	72	1.3633	1.3535
36	1.1590	1.1582	73	1.3696	1.3587
37	1.1641	1.1631	74	1.3760	1.3658

dye may be detected by suspending silk fibres in a neutral solution of the sugar, or one slightly acidified with hydrochloric acid.

Many samples of true Demerara sugar contain a trace of tin, due to the use of chloride of tin to fix the natural colouring matter.

Traces of ultramarine are sometimes to be found. This substance is added as a corrective to the yellowish colour of poorly refined sugar. It is detected by dissolving the sugar in water, when the ultramarine will sink to the bottom in fine particles.

MOLASSES OR TREACLE.

Molasses, treacle, and golden syrup are to be understood as practically synonymous names for the uncrystallizable syrup, in a greater or less state of refinement, usually obtained as a secondary product in the manufacture of sucrose. Molasses contains a considerable amount of sucrose, together with (in molasses from cane sugar) a good deal of invert sugar, and (in molasses from beet sugar) impurities such as raffinose and other organic substances. It should consist of partially hydrolysed sucrose, and should therefore contain practically no sugars, other than sucrose and invert sugar.

Four samples of each variety, analysed by the author, had the following composition :—

	Cane Sugar Molasses.				Beet Sugar Molasses.			
	Sucrose.	Glucose.	Water.	Ash.	Sucrose.	Glucose.	Water.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Green syrup	56·8	9·15	24·1	2·2	55·4	1·4	22·1	10·1
Treacle	38·1	17·2	25·1	2·8	52·1	0·8	20·9	9·9
Golden syrup	42·1	20·8	23·9	2·1	49·9	0·9	19·6	13·4
" "	39·5	23·5	25·2	1·4	56·1	1·6	23·0	11·8

According to Winter Blyth the ash of ordinary treacle is 8·21 per cent and the reducing sugars 12·92 per cent. It is to be noted, however, that in the more highly refined golden syrup, the percentage of sucrose is lower than in the green syrups and the proportion of reducing sugars correspondingly higher. Ling and Maclaren give the following as the sugar values of five samples of cane molasses :—

Sucrose (Copper Process).	Sucrose (Clerget).	Invert Sugar.
Per cent	Per cent	Per cent
40·2	40·6	17·9
34·8	34·8	17·1
28·7	27·8	11·7
35·3	34·9	15·3
65·6	66·0	7·2
		(doubtful sample)

Bodmer, Leonard and Smith give the following as representing the composition of pure golden syrups, or treacles :—

	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Water	16·0	13·8	16·2	16·3	20·1	16·5	16·9
Ash	2·0	8·2	4·1	2·1	7·9	2·0	1·8
Sucrose	33·3	32·4	25·8	34·1	40·8	28·4	34·9
Invert sugar	45·7	32·5	45·6	45·3	22·0	44·0	40·8
Other organic matter	3·0	3·1	8·3	2·2	9·2	9·1	5·6
Specific rotation	15°	17·5°	11·5°	13°	25°	12·5°	16°

The analysis of molasses is conducted on the same principles as that of ordinary sugars, the principal adulterant to be looked for being starch-glucose syrup, which is now often sold either slightly flavoured, or mixed with a little genuine molasses under the name "amber syrup".

Water.—This averages from 20 to 30 per cent, and is best determined by dissolving 10 grms. in water to make 100 c.c. and then drying 10 c.c. (= 1 gm. of the sample) in a platinum capsule containing recently ignited sand. This prevents a pellicle forming, which prevents the water from escaping.

Mineral Matter.—This is determined as described under cane sugar and averages from 2 to 6 per cent in cane molasses and from 9 to 13 per cent in beet molasses.

Sugars.—The reducing sugars may be determined by Fehling's solution in the usual manner. On inversion of a genuine molasses, the rotation will change from a dextro- to a laevo-rotation, whilst if much added glucose be present the dextrorotation will remain after inversion, as dextrose is not affected by the inversion.

In determining the copper reducing power, when necessary, the gravimetric process is preferable, as the solutions are somewhat dark. Ten c.c. of 10 per cent solution should be used with 20 c.c. of Fehling's solution. By heating with dilute acid to 68° for ten minutes, little but the sucrose is inverted, so that the difference between the two reducing powers will enable the sucrose to be approximately calculated. A third determination, after inversion of 10 c.c. of the solution with 40 c.c. of water containing 1.5 c.c. of strong sulphuric acid for three hours on a water bath, by which the dextrin and maltose are inverted, will enable the amount of these substances to be determined.

The presence of dextrin and maltose is strong evidence of adulteration with glucose syrup. Dextrin is indicated by a white precipitate being formed when 20 c.c. of alcohol is added to 2 c.c. of the 10 per cent solution of the syrup. Care must be taken, however, with this reaction as a turbidity is *sometimes* produced by pure samples.

The following figures will show the difference in the character of pure golden syrups and of those containing much glucose syrup:—

	Specific Rotation.	Reducing Sugars (as Glucose).		
		Original Syrup.	Inversion at 68° for 10 minutes.	Inversion at 100° for 3 minutes.
Pure syrup	+ 16° 30'	Per cent 44	Per cent 76	Per cent 77
" "	+ 14°	39.5	76.8	75
" "	+ 17°	35	69.5	71.8
Adulterated syrup	+ 70°	39	51	76
" "	+ 63°	40	59	77
" "	+ 92°	33.8	51	71.5

Bernard Dyer ("Analyst," xxv. 95) gives 42 as the K value which may be safely used for glucose syrup (i.e. the percentage of reducing sugars in terms of pure glucose taken as 100), and $+113^\circ$ as the specific rotatory power. He uses the following formulæ for calculating the percentage of glucose syrup present. He estimates the amount of sucrose present by a determination of the specific rotation before and after inversion (see Clerget's process, p. 132) and determines the cupric reducing power K. If P be the percentage of glucose syrup, R the specific rotation, $[a]_d$, of the original sample, and S the rotation due to the sucrose, then

$$P = \frac{0.206K + (R - S)}{1.217}$$

if angular degrees be used, and

$$P = \frac{0.31K + (R - S)}{1.83}$$

if percentage degrees are used, R here being the percentage reading before inversion and S the actual percentage of sucrose.

According to Leach the following formula gives approximate results (allowance being made for the average variable constituents present) when commercial glucose is present in molasses etc. :—

$$G = \frac{(a - S)100}{175}$$

where G is the percentage of commercial glucose; *a* the direct polarization on the sugar scale for normal weight, and S the percentage of sucrose as determined by Clerget's process.

The variable composition of both natural molasses or treacle, and of glucose syrup, prevents any absolutely accurate determination of the amount of each of these substances in a mixture, but approximate valuations may be made by a determination of the optical values of the syrup. Glucose syrup consists principally of dextrose, with some dextrin and maltose. These compounds being dextrorotatory, the optical rotation of the syrup at once affords a clue to the presence of adulteration with glucose syrup. The *average* specific rotation for sodium light of genuine golden syrup is about $+16^\circ$, and that of glucose syrup $+110^\circ$ (Bodmer, Leonard and Smith, "Analyst," xxiv. 252). So that if $[a]_d$ be the specific rotation of the sample, the approximate amount of glucose present is $\frac{100([a]_d - 16)}{100 - 16}$. Bodmer,

Leonard and Smith (loc. cit.) recommend the following process to be used in examining this syrup :—

Water.—This may be determined with sufficient accuracy by making a 10 per cent solution of the syrup, and taking the specific gravity of water as 1000, and deducting this from the observed specific gravity of the solution, and dividing the result by 3.86, the amount of solid matter in the 10 per cent solution is found. Thus if the specific

gravity be 1.032 (and it should not be much below this, the solid matter in the original syrup is $\frac{32}{3.86} \times 10$ or 82.9 per cent, the water being 17.1 per cent. Jones ("Analyst," xxv. 87) prefers the figure 4 as the divisor, which would give the water as 20 per cent. Bodmer, Leonard and Smith then clarify, if necessary, the ten per cent solution and take its rotatory power, from which the specific rotation is calculated from the usual formula $[\alpha]_d = \frac{100a}{lc}$, where l is the length of the tube in decimetres and c the number of grms. per 100 c.c. If a 200 mm. tube be used the observed angle has merely to be multiplied by 5.

The average specific rotation of pure golden syrup after inversion is -12° for sodium light (-14° for the transition tint). The approximate amount of glucose syrup may therefore be calculated, if the syrup is dextrorotatory after inversion, from the formula

$$P = \frac{([\alpha]_d \text{ after inversion} + 12) 100}{110 + 12}$$

Matthews and Parker ("Analyst," xxv. 89) calculate the sucrose in genuine golden syrup by taking the rotation of a 10 per cent solution before and after inversion with yeast (1 grm. to 50 c.c. at 52° for five hours—afterwards boiling to destroy bi-rotation). The sum of the readings in a 200 mm. tube (neglecting their signs) is multiplied by 10 and divided by 5.02 (the divisor for a 1 per cent sucrose solution in a 200 mm. tube when inverted). This gives the percentage of sucrose. For the calculation of the reducing sugars by fermentation processes and for the estimation of the dextrin and maltose the original paper should be consulted.

It is to be remembered that there are to be found in some samples of molasses—especially in beet molasses—optically active substances other than sugars. It is probable that little effect is caused by these on the ultimate sugar value determined by optical methods as the other active bodies fairly neutralize each other. Most of such optically active bodies are best precipitated by a little lead acetate and alcohol.

COMMERCIAL GLUCOSE.

Under the names of glucose or glucose syrup is usually sold a syrup, containing a large amount of dextrose, made by the hydrolysis of starch by acid. The principal variety is that imported from America, made from maize starch. Solid glucose (saccharum or saccharine) is also a regular article of commerce. Apart from the use of such products of the conversion of starch in the brewing and other industries, glucose syrup is frequently used as an adulterant of golden syrup, and of honey. Since the attention of analysts has been drawn to this, glucose syrup, coloured and slightly flavoured, is often sold under the name "amber syrup". Glucose has also a legitimate use in medicine, the British Pharmacopœia prescribing a syrup of glucose. The constituents of "glucose" of commerce include true dextrose,

dextrin, maltose and, often, a notable proportion of unfermentable carbohydrates to which the name gallisin has been given. Gallisin is probably a mixture of bodies (gluco-amylins) of the average specific rotation $[\alpha]_d$ = about $+82^\circ$. According to Valentin ("Journ. Soc. Arts." xx. 14, 404), the following represent the compositions of five samples of good quality commercial glucoses.

	1.	2.	3.	4.	5.
	Per cent	Per cent	Per cent	Per cent	Per cent
Dextrose	80.00	58.85	67.44	63.42	61.46
Maltose	none	14.11	10.96	23.50	13.20
Dextrin	none	1.70	none	none	none
Unfermentable carbohydrates	8.20	9.38	4.30	8.40	8.60
Mineral matter	1.30	1.40	1.60	1.50	1.60
Water	10.50	14.56	15.70	13.18	15.20

These were solid "glucose" of English, French or German make. The small proportion of dextrin present is inexplicable, especially when contrasted with the following analyses of Steiner:—

	1.	2.	3.	4.
	Per cent	Per cent	Per cent	Per cent
Dextrose	45.40	26.50	76.00	—
Maltose	28.00	40.30	5.00	42.60
Dextrin	9.30	15.90	—	39.80

The characters of numerous samples of glucose syrup examined in the author's laboratory are as follows:—

Specific gravity	1.400 to	1.4370
K value (in terms of dextrose)	40.5	" 66.8
$[\alpha]_d$	$+89^\circ$	" $+108^\circ$

For calculating the approximate amount of glucose syrup in golden syrup, Dyer, as stated above, uses the K value 42, and specific rotation $+113^\circ$. These values are safe to use, in so far as they give the mixer any benefit of the doubt. Dextrin is determined by precipitating 1 volume of a 25 per cent solution of the sample, by 10 volumes of 90 per cent alcohol, collecting the precipitated dextrin, washing it with alcohol and weighing on a tared filter.

According to experiments by Wiley, inversion by dilute acid reduces the specific rotation to about $+54^\circ$ showing that dextrose is the principal ingredient present after inversion. The K value becomes from 80 to 90.

If it be necessary to separate the glucose and maltose, the following scheme of analysis may be adopted:—

Water and Mineral Matter.—Dry about 1 grm. of the sample to

constant weight, and then ignite the residue. This will give the water, mineral matter and organic matter.

Reducing Sugars.—Ascertain the K value by reduction of Fehling's solution.

Specific Rotation.—Determine the specific rotation by using a 20 per cent solution.

Taking the specific rotations as follows: glucose + 53°; maltose + 139.2°; dextrin + 198°, the following formulæ can be deduced for ascertaining the percentage of maltose, glucose, and dextrin. Let M = percentage of maltose; G that of glucose and D that of dextrin. Then

$$(1) M = \left([a]_d - \frac{53 K + 198 (O - K)}{100} \right) \div 0.313$$

$$(2) G = K - 0.62 M$$

$$(3) D = O - G - M$$

where O is the percentage of organic matter, and K the usual cupric reducing power, and $[a]_d$ the specific rotation of the sample.

According to the Report of the Committee of the American Academy of Sciences, the following are the average compositions of solid and liquid "glucoses":—

	Solids.	Liquids.
	Per cent	Per cent
Water	1.4 to 17.6	14.2 to 22.6
Dextrose	72 „ 73.4	34.3 „ 42.8
Maltose	0 „ 3.6	0 „ 19.3
Dextrin	4.2 „ 9.1	29.8 „ 45.3
Ash	0.33 „ 0.28	0.32 „ 1.06

HONEY.

Although a saccharine substance is secreted by several species of the Hymenoptera, commercial honey is, in at all events nearly every case, the production of the bee, *Apis mellifica*.

Honey consists chiefly of a concentrated solution in water of various sugars, dextrose and levulose being the principal. In certain honeys cane sugar (sucrose) is present. According to Bell, there is present from 5 per cent to 10 per cent of a substance which is with difficulty hydrolysed to glucose, and which may be maltose. Mannite is also present, and up to 3 per cent or thereabouts of dextrin, principally in the form of achroo-dextrin. A small amount of wax is normally present, and flavouring matters, a small amount of mineral matter, and some pollen grains.

The average composition of a normal honey is as follows:—

	Per cent
Dextrose . . .	25 to 40
Levulose . . .	30 „ 45
Mannite . . .	about 1 „ 3
Mineral matter . . .	0.1 „ 0.3
Water . . .	15 „ 25
Sucrose . . .	0 „ 8 (11 per cent is found occasionally)

It is often stated that in normal honey the dextrose and levulose are present in about equal parts, but that when it has crystallized in the comb there will be an excess of levulose, and the honey is lævorotatory. It is said also that all honey will crystallize, and those remaining syrupy are adulterated. This however is not the fact, as many syrupy honeys are certainly genuine.

In the analysis of honey, a microscopic examination will afford useful information. Pollen grains, fungus spores, tiny fragments of wings, etc., are to be observed. This is sometimes of importance, as there are honeys, so called, to be found, which are entirely factitious, and the total absence of pollen grains would be a strong indication of adulteration. Starch is not present. The usual adulterants are other carbohydrates. The chief of these is ordinary glucose syrup, obtained by the hydrolysis of starch, or invert sugar. Cane sugar is sometimes used, and possibly molasses, but the strong taste of the last named renders it an improbable adulterant to-day.

Moisture.—The water of genuine honey varies from 15 to 25 per cent. It is best determined in the following manner: About 3 grms. of the honey are weighed in a shallow platinum dish, and 3 c.c. of absolute alcohol added, and the whole mixed to a thin solution, a weighed quantity of freshly burned sand (about 3 grms.) is then added, and the dish left for an hour or so on a water bath. A few c.c. of alcohol are again added, and the dish again dried to a constant weight.

Ash.—The ash of genuine honey should not exceed 0.3 per cent or at most 0.4 per cent. The ash of artificial or adulterated honey is often higher than this on account of the fact that starch glucose very frequently contains calcium sulphate. If the ash be higher than 0.3 per cent, it should be digested with water, filtered and tested with barium chloride. The presence of more than the faintest trace of sulphates is strong evidence of the adulteration with starch glucose. The ash of honey mixed with invert sugar is, however, usually very low.

The British Pharmacopœia allows 0.25 per cent of ash as a maximum for purified honey.

Carbohydrates.—The hydrolysis of starch to glucose is usually carried to the point at which iodine gives a red reaction with the product, so that erythrodextrin and amylo-dextrin are usually present. This affords a useful means of deciding whether starch glucose is present. For even if the starch glucose contain none of the two dextrins mentioned above, it will still contain bodies of a dextrinoid nature, which although not precipitated by alcohol, yield barium compounds insoluble in methyl alcohol. Natural honey gives no such precipitate in most cases, and even in the most unfavourable case, that of coniferous honey, not more than 2.5 per cent. For a qualitative determination, 5 c.c. of a solution containing 20 grms. of honey in 100 c.c., are shaken with 2 c.c. of a 2 per cent solution of barium hydroxide and 17 c.c. of methyl alcohol. The precipitate should be collected on a Gooch filter, washed with 10 c.c. of methyl alcohol, then with 10 c.c. of ether, and dried at 60° C. More than a trace of precipitate is suspicious, and if the honey is not a coniferous one,

anything over 2.5 per cent will indicate the addition of starch glucose. More than 2.5 per cent will certainly indicate this adulteration in all cases. Molasses, if present, is best detected by examining the honey for raffinose. Five c.c. of a 25 per cent solution of the honey is treated with 2.5 c.c. of solution of basic acetate of lead (30 per cent), and 22.5 c.c. of methyl alcohol. Pure honey gives about 0.5 per cent to 1.5 per cent of precipitate, whilst molasses gives 50 per cent to 70 per cent. The specific gravity of pure honey is from 1.4150 per cent to 1.430 per cent, rarely up to 1.440 per cent.

The most important method of examination, however, is the determination of the optical rotation of the honey, both before and after inversion. It is to be noted, however, that in any such determination, the solutions must be allowed to stand for several hours, before the reading is taken, as they exhibit some amount of bi-rotation. The specific rotatory power S , is calculated from the formula

$$S = \frac{100a}{lc}$$

where a is the observed angle of rotation in a 100 mm. tube, l is the length of the tube in decimetres, and c is the number of grammes of substance in 100 c.c. A 200 mm. tube is most convenient for the reading, when, if the observed angle is used in the above formula, the value of l becomes two. The usual limits given for honey are not strictly correct, as since it has become known that cane sugar is a normal constituent of some honeys, a wider limit is necessary. Taking 20 per cent of water as an average for honey, the following adulterants give the specific rotatory powers before and after inversion. The figures for honey are also added:—

	Cane Sugar and 20 Per cent of Water.	Invert Sugar and 20 Per cent of Water.	Glucose Syrup.	Genuine Honey.
Original substance	+ 53.2°	- 18.4°	+ 90° to 102°	+ 5° to - 8°
After inversion ¹	- 19.5°	- 18.4°	+ 45° „ 50°	hardly altered

It is to be remembered that most honey has a specific rotation within the limits + 3° and - 3°, but that where cane sugar is normally present, as is undoubtedly the fact with certain honey, the rotation may be + 5° or a trifle over for the honey, and naturally, inversion will alter this figure to even - 1° or thereabouts. It is also true that where a honey has crystallized in the comb, it may be more highly laevorotary than even - 8°, although this is rare. So that unless a wide difference from the above limits is noted, care must be exercised in forming an opinion. For example, a strong dextrorotation, reduced on inversion to a laevorotation, would indicate the presence of cane sugar. Glucose is indicated by a very high dextrorotation, which is

¹ Inversion by heating a 20 per cent solution with 10 per cent of hydrochloric acid for ten to twelve minutes to 70° C. The specific rotation is for sodium light.

reduced to about one half by inversion, but the heating should be for twenty minutes to half an hour in this case. Mixtures of cane and invert sugar or invert sugar and glucose may have correct initial rotations but a mixture of invert sugar and glucose of specific rotatory power about that of honey will undergo a greater change on inversion than most honey does.

It is generally understood that honey derived from flowers is always lævorotatory, and that from conifers dextrorotatory. This is not correct, however, as it is easy to produce a dextrorotatory honey by artificial feeding of the bees.

The optical rotation of the honey after fermentation by yeast is a figure sometimes determined, but as it takes considerable time, and possesses no advantage over inversion by means of acids, it is very rarely, if ever, resorted to.

Hænle ("Analyst," xvi. 79) states that if a solution of one part of honey in two parts of water (in the case of a lævorotatory honey) be dialysed for twenty-four hours, and the solution remaining in the dialyser be then examined, it will never have become dextrorotatory, unless glucose be present, when it will be found to be dextrorotatory.

Clerget's process (see under sugar) will give a pretty accurate determination of the amount of cane sugar present.

The reducing power of honey when boiled with copper oxide (v. p. 124) affords considerable information as to the purity of the sample. The following are the cupric oxide reducing values (K) of the usual adulterants, that of dextrose determined gravimetrically being taken as 100:—

	Cane Sugar and 18 Per cent Water.	Invert Sugar and 18 Per cent Water.	Glucose Syrup.	Genuine Honey.
Original value	0	82	50 to 55	60 to 80
Value after inversion with acid	86.3	82	80 „ 85	little altered

Determined by Fehling's solution pure honey should give a result equivalent to about 60 to 80 per cent of glucose (see under sugar).

Sieben ("Analyst," x. 34) recommends the determination of the optical rotation of the fermented honey, which if pure is nearly 0°, whereas with starch-glucose it is highly dextrorotatory. The author has found that no information is yielded by this, that is not given by inversion with acid. The process recommended by the same author depending on the determination of carbohydrates which do not reduce sugar involves several unwarrantable assumptions and is not reliable.

According to Ditte ("Zeit. Untersuch. Nahr. Genussm." 1909, 18, 625 - 649) the genuineness or otherwise of a sample of honey is best ascertained by estimating the total nitrogenous matter precipitated by tannin, and the amount of cane sugar present. The last mentioned may be calculated from the polarization of the honey before and after inversion. The results obtained on the estimation of the amounts of

total solids, invert sugar, acidity, and ash present in the sample affords little evidence of the presence or absence of artificial honey. The following figures were obtained on the analyses of fifty-two samples of honey, and from the results these are classified as pure honey (twenty-four samples), artificial honey (six samples), honey of suspicious quality (four samples), and adulterated honey (eighteen samples):—

	Pure Honey.			Artificial Honey.			Honey of Suspicious Quality.			Adulterated Honey.		
	Minimum Per cent.	Maximum Per cent.	Average Per cent.	Minimum Per cent.	Maximum Per cent.	Average Per cent.	Minimum Per cent.	Maximum Per cent.	Average Per cent.	Minimum Per cent.	Maximum Per cent.	Average Per cent.
Water	13.86	20.80	17.18	15.60	20.42	18.90	16.26	18.52	17.57	12.62	19.80	16.64
Invert sugar	68.10	76.97	72.33	56.88	71.74	63.83	72.04	78.06	73.84	69.84	76.40	73.81
Acidity (as formic acid)	0.043	0.153	0.087	0.018	0.095	0.052	0.040	0.098	0.073	0.031	0.068	0.054
Cane sugar	0.160	7.550	2.890	3.250	15.730	8.820	0.500	3.960	2.650	1.260	8.790	8.420
Ash	0.063	0.624	0.190	0.076	0.250	0.167	0.066	0.286	0.160	0.045	0.324	0.105
Total protein (N x 6.25)	0.254	0.636	0.398	0.114	0.173	0.147	0.219	0.272	0.244	0.135	0.289	0.185
Polarization of 25 Per cent Honey Solution in 200 Mm. Tube.												
Before inversion	+1.25	-9.54	-5.50	+9.45	-3.71	+4.25	-5.31	-6.39	-5.77	-4.36	-7.50	-6.20
After "	-1.19	-10.32	-6.57	+7.73	-8.18	-2.76	-6.61	-7.43	-6.98	-6.80	-8.35	-7.67
Vol. of tannin precipitate in c.c.	0.90	3.95	1.49	—	—	—	0.70	1.00	—	—	0.45	0.28

The tannin precipitate is obtained by placing 10 c.c. of filtered 20 per cent honey solution in a graduated tube, adding 35 c.c. of water, and then 5 c.c. of 0.5 per cent tannin solution. The contents of the tube are well mixed, allowed to stand for twenty-four hours, and the volume of the precipitate then read off; in the case of pure honey the volume of the precipitate is never less than 0.9 c.c.

SUGAR OF MILK.

Lactose or milk sugar, is a sugar prepared from the whey of curdled milk, and is employed, *inter alia*, to a very large extent in the manufacture of infant foods. It is official in the British Pharmacopœia, that authority prescribing that it should have the following character:—

It contains 1 molecule of water of crystallization, having the formula $C_{12}H_{22}O_{11}H_2O$. It is soluble in 7 parts of cold water and in about 1 part of boiling water. It should not yield more than 0.25 per cent of ash. One gram. dissolved in 10 c.c. of water, to which three drops of decinormal solution of sodium hydroxide have been added, give a red colour with phenol-phthalein. The water of crystallization is driven off by heating to 130° to 135° for some time.

In general, these characters, together with the optical values of the sample are sufficient to determine the purity of the sample.

Lactose exhibits the phenomenon of bi-rotation (see p. 128). In its stable condition, after standing for a time in solution, the specific rotation is $[\alpha]_d = +52^\circ$ for the crystals or $+55.8^\circ$ for the anhydrous sugar.

On hydrolysis by boiling with 20 per cent acid for several hours, it is converted into galactose and probably another sugar, which have an average specific rotatory power of $[\alpha]_d = +67.5^\circ$. (It is to be remembered that 1 molecule of lactose combines with 1 of water in yielding 2 molecules of six-carbon sugars.)

Lactose readily reduces Fehling's solution, 10 c.c. of the latter requiring 0.0685 gram. of lactose; or, if a gravimetric process be employed, 1 gram. of cupric oxide = 0.6153 grms. of anhydrous lactose.

In estimating lactose by means of Fehling's solution, the greatest accuracy is obtained, according to Muter, by first ascertaining the approximate amount required, and very rapidly bringing the Fehling's solution and the sugar together when both are boiling.

The determination of lactose in milk is described on p. 55 under milk.

It may here be convenient to give the analyses of a few typical infant foods.

	H ₂ O.	N × 6.25.	Fat.	Carbohydrates mostly Lactose with some Starch.	Ash.	P ₂ O ₅ .
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1.	2.0	10.7	18.6	66.6	3.95	—
2.	11.29	10.43	1.10	75.62	0.96	0.29
3.	5.08	9.67	0.34	86.34	2.02	0.06
4.	4.27	13.2	1.70	79.85	1.09	0.12
5.	7.06	8.70	1.38	81.54	0.64	0.06
6.	5.81	10.79	1.06	78.61	0.91	0.86

MALTOSE AND MALT EXTRACT.

Maltose is an important sugar which, although it does not come before the analyst as such very often, is of interest and importance, and may here be considered, together with extract of malt. The action of diastase on starch matters, such as rice, malt and various grains, results in a mixture of maltose and dextrin, both of which are converted into dextrose by hydrolysis with acids. To completely hydrolyse maltose by means of dilute acid, it is necessary to allow the reaction to proceed for three to four hours at 100° C. Neither diastase nor invertase hydrolyse maltose, hence a determination of sucrose in the presence of maltose is possible.

Maltose is bi-rotatory, and all solutions of this sugar should be kept for several hours before being examined polarimetrically. The specific rotatory power of anhydrous maltose varies slightly with the concentration, but for all practical purposes $[\alpha]_d$ may be taken as +138°.

Maltose reduces Fehling's solution, the K value, however, being only 62. Ten c.c. of Fehling's solution oxidize 0.081 grm. of maltose. If a gravimetric estimation be used, the $\text{CuO} \times 0.7314$ gives the amount of maltose.

Extract of Malt.—A thick viscous extract of malt is now a commercial article prepared on a very large scale by the evaporation of an infusion of malt at a very low pressure and at a sufficiently low temperature not to destroy the properties of the diastase. Extract of malt is used largely in medicine, either alone, or more often in combination with oils, such as cod liver or olive oil.

In examining an extract of malt, the most important determination is its diastatic power, as it owes its value principally to its power of converting starch into easily assimilable carbohydrates. Diastase is an unorganized ferment formed during the germination of barley and other grains. In converting the starch, diastase changes $\frac{2}{3}$ of it into maltose and $\frac{1}{3}$ into dextrin, which is slowly further converted into dextrose. According to Ling and Baker, the starch is gradually converted into maltose and a series of malto-dextrins of gradually decreasing molecular weight and optical activity, but of higher reducing powers.

The composition of pale malt is shown by the following analyses by O'Sullivan, and this of course is in close relationship to the composition of the solid matters of extract of malt:—

	I.	II.
	Per cent	Per cent
Starch	44.15	45.13
Other carbohydrates (of which 60 to 70 per cent are fermentable sugars)	21.23	19.39
Cellular matter	11.57	10.09
Fat	1.65	1.96
Albumenoids	13.09	13.80
Ash	2.60	1.92
Water	5.83	7.47

The complete examination of extract of malt should include the following determinations:—water, total solid matter, mineral matter, sugars, diastatic value (and, of less importance, dextrin, proteids and phosphoric acid).

Water and Total Solid Matter.—These may be determined by drying 10 c.c. of a 10 per cent solution in a platinum dish on recently ignited sand. Approximate accuracy may be obtained by calculating from the specific gravity of a 20 per cent, i.e. 20 grms. in 100 c.c., solution of the extract in water. The following formula will give the percentage of solid matter:—

$$(D - 1000) \times 1.3$$

Where D is the specific gravity (water = 1000) at 15.5°.

A good malt extract should not contain less than 70 per cent of solid matter, as diastase does not keep well in weaker solutions unless a preservative be added which usually destroys the activity of the diastase. A thin fluid extract of malt exists which often contains alcohol.

Mineral Matter.—This is best determined on 5 grms. of the sample—which is nearly dried in an air oven at 110° and then carefully ignited. The carbonized mass requires breaking to get a pale ash within any reasonable time.

Sugars.—The reducing sugars are determined by using 2 c.c. of a 10 per cent solution and 30 c.c. of Fehling's solution, preferably using the gravimetric process. Cane sugar is determined by making 20 c.c. of a 10 per cent solution up to 100 c.c., warming to 55° C., and adding about 0.2 gm. of yeast (dried between blotting paper). 0.1 per cent of thymol should be added to prevent fermentation. The whole is allowed to digest at 55° for four hours (the loss by evaporation being made up) and the reducing sugars are then determined in 10 c.c. of the filtered liquid. The difference in this and the preceding determination is due to glucose formed by inversion of the cane sugar, which is thus easily calculated.

In most cases it is sufficient to assume that the whole of the original reducing sugar consists of maltose, but this is not strictly accurate, as about 12 per cent to 15 per cent consists of glucose. If it be necessary to determine the amount of glucose present—and more than about 16 per cent should be viewed with suspicion as indicating added glucose—the only practicable manner is to determine all the constituents other than reducing sugars (i.e. the cane sugar—dextrin, proteids water and ash) and call the difference of these and 100 the total reducing sugars = S. Let W = the weight of cupric oxide precipitated by 100 grms. of extract.

But 1 grm. of maltose precipitates 1.37 grms. of CuO, and 1 grm. of glucose precipitates 2.21 grms. of CuO.

Let the percentage of maltose = M, and of glucose = G.

Then :—

$$\begin{aligned} M + G &= S \\ \text{and} \\ 1.37 M + 2.21 G &= W. \end{aligned}$$

From these two equations, the values of M and G may be calculated. The average amount of maltose in a well-prepared extract of malt is over 50 per cent, and of glucose, according to Ling, from 12 per cent to 20 per cent.

Diastatic Value.—The usually adopted method of determining the diastatic value of malt extract is that of Lintner, and the results are expressed in “degrees Lintner”. On Lintner’s scale, the diastatic capacity of a malt or its extract is regarded as 100, when 0.1 c.c. of a 5 per cent solution converts enough starch in an hour at 70° F. to reduce 5 c.c. of Fehling’s solution.

The valuation is carried out as follows: Soluble starch is prepared by treating ordinary starch (e.g. potato starch) with 7.5 per cent hydrochloric acid for seven days at ordinary temperatures. It is washed with cold water until every trace of free acid is removed, when it is dried. It is then soluble to a clear solution in hot water.

A 2 per cent solution of soluble starch is prepared, and cooled. To ten test tubes are then added 10 c.c. of the solution and the tubes numbered 1 to 10. A 5 per cent solution of the malt extract is then made and quantities of 0.1 c.c., 0.2 c.c. and so on up to 1 c.c. are added to the tubes 1 to 10. They are now allowed to stand for one hour in a water bath at 70° F. and then 5 c.c. of Fehling’s solution are added to each tube. The tubes are shaken up, and stood in boiling water for ten minutes and the copper oxide allowed to subside. The colours of the tubes are then noted. Some will be found to be yellow, all the copper being reduced, and others will still be blue. If tube 5 be completely reduced and tube 6 be still slightly blue, the amount of the 5 per cent solution necessary to convert the starch required to reduce the Fehling’s solution is obviously between 0.5 c.c. and 0.6 c.c. The amount of blue colour left in the tube can be fairly well judged by a little practice—so that the second place of decimals can be gauged with fair accuracy. If the test tube gives 0.55 as the amount necessary, then the apparent diastatic power will be

$\frac{0.1 \times 100}{0.55}$ or 18.2° , subject, however, to a correction for the amount of reducing sugar, actually present in the small amount of malt extract used in the experiment. This is determined by a previous estimation, and a deduction made. The deduction usually given is that deduced from a determination of the "Lintner value" of the reducing sugars of the extract, that is, for example, if 1.5 c.c. of the 5 per cent solution reduced 5 c.c. of Fehling's solution, the Lintner value would be $\frac{0.1 \times 100}{1.5}$ or 6.6° . But as a different amount of the malt solution is used in each tube, this correction is obviously inaccurate. The proper correction is to deduct from the 5 c.c. of Fehling's solution reduced, the number of c.c. that would be reduced by the amount of sugars in the quantity of malt extract in the completely reduced tube. If, for example, 0.5 c.c. of malt extract solution were judged to be the proper quantity, and a previous experiment showed that this amount contained sugars sufficient to reduce 1.5 c.c. of Fehling's solution, then the true amount of Fehling's solution reduced through the diastatic action is only 3.5 c.c., and the observed apparent diastatic value must be multiplied by $\frac{3.5}{5}$. Very high grade malt extracts will show a Lintner value of well over 50 but these are rare.

If the diastatic value is very high it may be necessary to use a weaker solution of the extract of malt and make the corresponding calculations.

Gadd has found that good commercial extracts of malt will convert $2\frac{1}{2}$ times their weight of potato starch (not soluble) at 100°F. , in from four and a half minutes to fifteen minutes.

Takamine suggests the use of standard solutions of taka-dia-*stase* as a means of determining the value of malt preparations. Taka-dia-*stase* is prepared from a fungus cultivated on wheat bran and its diastatic value is determined by Lintner's method and a standard is so fixed. The details of the process are as follows:—

*Standard Solution of Taka-dia-*stase*.*—A 10 per cent solution of the standardized ferment in distilled water is made.

Starch Solution.—Fifty grms. of neutral potato starch are made in the usual manner into a 5 per cent solution, by rubbing the starch down with cold water and then boiling for two minutes.

Iodine Solution.—A 1 per cent solution of iodine in water (1 grm. of iodine and 2 grms. of KI are dissolved in a little water and made up to 100 c.c.).

Eight test vessels are used, to each of which is added 100 c.c. of the starch solution, the whole being kept in warm water at 104°F. Into the first test glass 1 c.c. of the liquid to be tested is added; into the second, 1 c.c. of standardized taka-dia-*stase* solution, into the third 2 c.c. of the solution, and so on. All the liquids are well stirred and when the starch is limpid—the conversion being very rapid with taka-dia-*stase*—in about ten minutes, a drop of each is mixed on a tile with

a drop of iodine solution and the colour of the sample to be tested is matched with the nearest of the other seven—which range from blue to purple brown. This gives a comparison of the diastatic value of the sample in terms of the standard taka-diastase solution, which has itself been standardized in terms of starch converted.

The author does not find that nearly so concordant results are obtained by this method as by Lintner's.

Ling ("Analyst," xxix. 244) gives the following figures for a number of commercial extracts:—

	1.	2.	3.	4.	5.	6.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Specific gravity at 15.5°	1.3957	1.3951	—	—	1.4084	1.3778
Maltose	31.1	30.9	24.8	27.4	34.2	25.2
Dextrose	17.2	18.2	22	19.1	12.5	20
Dextrin	9.8	8.6	10	9.8	9.9	6.7
Ash	1.45	1.49	1.58	1.64	1.34	1.64
Water	24.30	24.67	27.36	24.84	24.38	29.52
Diastatic value . . .	30.8°	27.2°	32.3°	25.6°	39.2°	46.5°
Specific rotatory power	91.8°	90.5°	84.2°	86.8°	94.5°	81.1°

Harrison and Gair ("Chemist and Druggist," 1906, II. 180) give the following analyses, which are of interest as many of them represent well-known brands of malt extract, and it is to be noted that the diastatic value is not expressed in the conventional manner. The methods of analysis adopted for those results are given below, including the determination of diastatic value:—

Sample.	Total Solids Per cent.	Maltose Per cent.	Proteids Per cent.	Diastatic Value.	Remarks.
I	73.2	65.4	7.0	468	—
II	79.8	64.4	5.0	346	—
III	69.8	58.5	4.1	356	—
IV	77.0	54.0	3.6	10	—
V	72.3	52.1	3.8	15	—
VI	95.9	82.1	5.7	89	Solid extract
VII	76.8	66.0	5.4	96	—
VIII	74.3	62.5	5.2	65	Considerable salicylate present
IX	73.0	47.1	3.8	17	9.5 per cent of cane sugar present
X	66.2	49.7	3.9	0	—
XI	78.7	74.2	5.5	268	High maltose figure probably due to glucose
XII	64.9	58.8	3.9	0	—
XIII	73.9	63.6	6.6	137	—

Total Solids.—Twenty grms. of extract were dissolved in water and made up to 100 c.c., and the specific gravity of the solution de-

terminated. The percentage of total solids in the extract was found by the formula :—

$$\text{T. S.} = \frac{\text{specific gravity} - 1000}{3.92} \times 5.$$

Maltose.—Five c.c. of the solution as used for specific gravity were diluted to 100 c.c.; then 10 c.c. of Fehling's solution were diluted with 40 c.c. of water and boiled in a porcelain beaker, and the malt solution run in from a burette until exactly all the copper was reduced. Since 10 c.c. of Fehling's is reduced by 0.0805 of maltose, the percentage of maltose in the extract is given by the expression $\frac{805}{m}$

where m stands for the number of c.c. used.

Proteids.—Total nitrogen was determined by the Kjeldahl-Gunning method and the result multiplied by 6.3 was taken as proteid.

Diastase.—Dr. H. A. D. Jowett's excess of starch plan was followed, potato starch being used. An amount of starch is taken containing 1 grm. of the anhydrous substance, mixed in a mortar, with a few c.c. of cold water and poured into 65 c.c. of boiling water. The mortar is rinsed with a little more water to make 15 c.c. in all, or a total of 80 c.c. of mucilage, which is boiled for about a minute to ensure complete gelatinization. The mucilage is then cooled to 46° C., and to it is added 20 c.c. of the same solution of malt extract as was used for the titration of maltose. This solution contains 1.0 of extract in 100 c.c., so that the quantity of extract taken to digest the starch is 0.2 grm. The mixture is then kept at 40° C. for exactly half an hour, then boiled to stop the action going further. The liquid is then cooled and adjusted to measure 100 c.c., and 100 c.c. of Fehling's solution is titrated with this as described under maltose. From the maltose found it is necessary to deduct the maltose introduced into the extract. The calculations may be combined by the use of the following formula :—

$$\text{Weight of anhydrous starch completely converted} = 1.184 \left(\frac{8.05}{n} - \frac{1.61}{m} \right)$$

where n is the number of c.c. used in the last titration, m (as above) is the c.c. used in the former maltose titration, and 1.104 is the factor $\frac{100}{84.4}$ for calculating maltose into starch.

The diastatic power may be conveniently expressed numerically by the weight of starch converted by 1 part of the extract, or, to avoid fractions, by 100 parts. The figures given in the table for diastase represent accordingly the percentage of starch which the extract is capable of completely converting in half an hour at 40° C. Since 0.2 grm. is the weight of extract taken for the test, the above result must be multiplied by 500, or

$$\text{Diastatic value} = 592 \left(\frac{8.05}{n} - \frac{1.61}{m} \right).$$

Ling ("Chemist and Druggist," 1910, II. 52) has criticised Harrison's

results in a very able paper. He shows that various considerations necessitate a modification in the formula advocated by Harrison and Gair, and the importance of his work justifies the reproduction of this paper *in extenso*.

He points out that it is well known that when the reactions which most enzymes bring about are graphically expressed on a co-ordinate system, they appear, in their initial stage, as linear functions of the time or mass; also that after a certain point has been reached, the curves representing them are logarithmic; or, in other words, the reactions then follow the law of mass action. It was the recognition of these facts by J. Kjeldahl ("Comptes Rend.," Carlsberg, 1879, **1**, 109) with regard to the reaction between malt diastase and starch paste that rendered diastasimetry—or, to speak more correctly, the measurement of diastatic power—possible.

Kjeldahl showed (*loc. cit.*) that when time and temperature are constant, the starch-hydrolysing power of a malt or malt extract is directly proportional to its mass; provided that not more than some 40 per cent of maltose, estimated by the cupric-reduction method, and calculated on the original starch, is produced. It is after this point that the reaction becomes logarithmic. Kjeldahl proposed a method of measuring the diastatic power of malt based on these observations, but it was not altogether satisfactory, and a modification was proposed by C. J. Lintner in 1887 ("J. pr. Chem.," 1887, [2], **34**, 375). In this method soluble starch prepared by the action of 7.5 per cent hydrochloric acid on potato starch was used instead of ordinary potato starch as substrat. Still another modification of the method was devised by Ling in 1900 ("J. Fed. Inst. Brewing," 1900, **6**, 355), and this is now almost exclusively employed by brewers in the United Kingdom, it having been adopted by the Malt Analysis Committee of the Institute of Brewing (see "J. Inst. Brewing," 1906, **12**, 6). It consists in extracting 25 grms. of finely ground malt with 500 c.c. of distilled water at 21° C. for an hour, and allowing a portion of the filtrate to act at 21° C. for an hour on 100 c.c. of a 2 per cent solution of soluble starch prepared by Lintner's method. After this 10 c.c. of N/10 caustic potash is added to stop further diastatic action, the liquid is made up with water to 200 c.c., and titrated against 5 c.c. of Fehling's solution. The diastatic power is calculated on Lintner's standard, the value of 100 being assigned to a malt 0.1 c.c. of the extract of which, after acting on starch solution under the above conditions, exactly reduces 5 c.c. of Fehling's solution. The results are calculated by the formula

$$D = \frac{1000}{xy},$$

in which D is the diastatic power of the malt, x is the number of cubic centimetres of malt extract contained in 100 c.c. of the fully-diluted starch-conversion liquid, and y equals the number of cubic centimetres of the same liquid required for the reduction of 5 c.c. of Fehling's solution. It is important that the volume of malt extract employed for the conversion shall be less than would produce a reducing power

exceeding the limit of Kjeldahl's law (*vide ante*). In the case of concentrated malt extracts an aliquot portion of a 5 per cent solution would be employed.

Harrison and Gair's method, which has since been adopted by the "Pharmaceutical Codex," aims at determining diastatic power by measuring the amount of starch dissolved in half an hour at 40° C. by a standard solution of malt extract. A weight of potato starch corresponding with 1 grm. of the anhydrous substance is made into a paste with a convenient quantity of water. The paste is rinsed into 100 c.c. measuring flask with water, so that its total volume does not exceed 80 c.c. After cooling to 40° C., a solution, at the same temperature, of 0.2 grms. of the malt extract in somewhat less than 20 c.c. of water is added, and the mixture is then kept at 40° C. for half an hour, when it is boiled to arrest further diastatic action, cooled, made up to 100 c.c. and titrated against 10 c.c. of Fehling's solution. From the maltose found, that present in the amount of malt extract used is deducted, this being estimated by a separate experiment; the diastatic power is expressed by the weight of starch converted by 100 grms. of the extract.

In order to understand the formula by which the results may be calculated on Harrison and Gair's assumptions, it will be necessary to consider a few additional points in the literature. It was shown by H. T. Brown and J. Heron ("C. S. Trans." 1879, **35**, 634) that at temperatures below 60° C., starch paste is rapidly hydrolysed by malt diastase until the products show the following constants, calculated on the total solid matter in the solution:—¹

$[\alpha]_d$	150°
R (percentage of apparent maltose)	80

Beyond this point further change takes place comparatively slowly (see also Brown and Millar, "C. S. Trans." 1899, **75**, 315).

Since every grm. of starch hydrolysed yields 1.05 grms. of apparent maltose, 80 per cent of maltose will correspond with an increase in solid matter on the original starch of 84 per cent and the factor necessary for calculating maltose into starch will be $\frac{100}{84} = 1.19$.²

The simplest formula by which the results can be calculated on these assumptions is therefore

$$\Delta = \frac{1.19M}{E},$$

in which M is the maltose formed per 100 grms. of starch and E is the weight (in grms.) of malt or malt extract used. The formula of

¹ These values are calculated from the constants given by Brown and Heron (*loc. cit.*), which are $[\alpha]_{57.86} 162.6^\circ$ $\kappa_{57.86}$ (percentage of apparent glucose expressed on 100 grms. of the total solids of the solution, estimated by the 3.86 divisor) 49.3.

² The factor used by Harrison and Gair is

$$\frac{100}{84.4} = 1.184.$$

Harrison and Gair is open to the objection that it assumes a constant maltose titre for Fehling's solution.

Since the amount of starch hydrolysed is measured by the amount of maltose produced, it is obvious that the results cannot be accurate unless this amount of maltose does not exceed 40 per cent of the starch, in accordance with Kjeldahl's law. Harrison and Gair, however, ignore this law entirely.

More recently Harrison ("Pharm. J.," 1909, **82**, 388) has recognized that the method is faulty with highly diastatic malt extracts, and in reference to these he says:—

"Clearly no higher results could be obtained than the conversion by the extract of five times its own weight of starch (or a diastatic power of 500), since no more is present. If it can convert more than this, the best plan is to reduce the quantity of extract, keeping all the other quantities constant. But if by proceeding in this way two extracts were found to give values of, say, 450 (using 0.2 gm. of extract) and 550 (using 0.1 gm. of extract), these two figures would not represent their respective powers with as near an approach to quantitative truth as if they were, say, 100 and 150, since in the first case nine-tenths of the starch present would have been used up, and in the second only eleven-twentieths, and the rate of conversion becomes less as the excess of starch becomes less. I find it best, therefore, if an extract gives a diastatic value of over 250, to repeat the test with only half the quantity of extract; if a diastatic power of over 750 is found, to reduce the extract to one-fourth the original quantity—i.e. to 0.05 gm. This allows for values up to 2000 being recorded, and, if necessary, the quantity can of course be further reduced: in each case water is added to make the total liquid in which conversion occurs measure 100 c.c."

In carrying out the experiments now to be described Ling found it more convenient, instead of weighing out separate quantities of starch and malt extract, to take definite volumes of starch paste and of malt extract solution. In the following experiment 50 c.c. of a starch paste containing 2 grms. of anhydrous starch per 100 c.c. was treated with increasing quantities of a 1 per cent solution of malt extract. The mixture was kept at a constant temperature of 40° C. for half an hour, at the end of which time 0.5 c.c. of normal caustic potash was added to arrest further diastatic action,¹ the volume made up to 100 c.c., and the liquid titrated against 10 c.c. of Fehling's solution by the method devised by Ling and Rendle ("Analyst," xxx. 182).

The values given under the various columns in the tables are as follows:—

A is the number of c.c. of 1 per cent malt extract solution taken.

M is the maltose produced, calculated as a percentage on the original starch.

E is the weight (in grms.) of malt extract taken.

¹ This method of arresting diastatic action is far preferable to that of boiling the solution, since the latter is not sufficiently rapid and may cause an error.

Δ is the diastatic power of the malt on Harrison and Gair's standard.

D is the Lintner value for half an hour's action at 40° C., calculated by Ling's formula.

EXPERIMENT I.

No.	A.	M.	1·19M.	$\Delta = \frac{1·19M}{E}$	$D = \frac{1000}{xy}$
1	1	13·88	16·52	1652	168
2	2	29·61	35·23	1762	177
3	3	42·57	50·66	1689	170
4	5	59·92	71·30	1426	—
5	10	68·19	81·14	811	—
6	20	67·63	80·48	402	—

EXPERIMENT II.

This was carried out in precisely the same manner as Experiment I., but a solution of soluble starch prepared by Lintner's method was used instead of starch paste.

No.	A.	M.	·19M.	$\Delta = \frac{1·19M}{E}$	$D = \frac{1000}{xy}$
1	1	15·57	18·51	1851	185
2	2	31·26	37·19	1859	185
3	3	46·33	55·13	1837	183
4	5	58·48	69·58	1391	—
5	10	61·21	72·84	728	—
6	20	62·99	74·94	374	—

EXPERIMENT III.

Twenty-five grms. of a low-dried distiller's malt was extracted with 500 c.c. of water at 21° C. for an hour, as in the Lintner method. Increasing volumes of the filtrate were then allowed to act on a solution of soluble starch for half an hour at 40° C. as in Experiments I. and II.

No.	A.	M.	1·19M.	$\Delta = \frac{1·19M}{E}$	$D = \frac{1000}{xy}$
1	0·25	14·43	17·17	1373	133
2	0·50	29·15	34·68	1387	135
3	0·75	41·94	50·00	1333	131
4	1·0	49·74	59·18	1184	116
5	2·0	60·77	72·30	723	72
6	3·0	63·31	75·34	502	49
7	5·0	63·91	76·05	304	30
8	10·0	66·99	79·72	159	16
9	25·0	69·24	82·39	66	8

It will be seen from these results that Harrison's latest suggestions, whilst they tend to increase the accuracy of the method, do not eliminate entirely the error inherent in the formula of Harrison and Gair. Thus, as Ling's results show, with malt extracts having a diastatic power of 1700 to 1800 on Harrison and Gair's scale, if a weight of extract of 0.05 gm. were taken (as Harrison suggests for extracts having a value up to 2000), the results would not be accurate, because under these circumstances more than 40 per cent of maltose, calculated on the original starch, is produced. But without a knowledge of Kjeldahl's empirical law, it is possible to fix the limit of accuracy of any method of diastasimetry by diminishing the weights of diastatic substance taken until two experiments with different weights give uniform results. With a malt extract of diastatic power 2000 on Harrison and Gair's scale, it will be necessary, in order to attain accuracy, to take a smaller weight than 0.05 gm. In the case of a highly diastatic malt extract, three experiments should be made with 0.05, 0.03, and 0.01 gm. of the sample respectively; those coming within the limit of Kjeldahl's law would give identical values.

From the last column in the tables, it will be seen that the Lintner value, calculated by Ling's formula, under the conditions of Harrison and Gair's method—that is to say, action of the diastase on starch paste or on soluble starch for half an hour at 40° C.—is approximately one-tenth that of the value on Harrison and Gair's scale. It must be remembered, however, that in the Lintner method action is allowed to take place for an hour—or, in other words, for twice the period prescribed by Harrison and Gair; consequently the Lintner value at 40° C. will be approximately one-fifth the Harrison value. This is a pure coincidence, since the basis of each of the methods is quite distinct.

Ling points out that Harrison and Gair's method is based on the assumption that the so-called stable equation of Brown and his co-workers is an absolute constant, which it certainly is not, the value of *R* (*vide ante*) varying according to the diastatic power of the sample of malt or malt extract used.

In conclusion, Ling urges that the scale employed by Harrison and Gair should for all purposes be replaced by that of Lintner, since the former records values to four places, thus beyond the limit of accuracy of the Fehling method, which, as he has shown elsewhere ("Analyst," xxxiii. 163), is, even in its most accurate form, only 1 in 300. Values of 1000 and upwards can therefore only tend to mislead manufacturers.

Squire advocates the following method for the determination of diastatic value. Two c.c. of a solution of iodine (about 0.2 gm. per litre) are run into each of twelve test tubes. A 5 per cent solution of the extract, and a 1 per cent solution of starch, well boiled, are made. These solutions are warmed to 100° F., and 50 c.c. of the starch are placed in a beaker kept in water at 100° F. To this 10 c.c. of the solution of the extract are added. At the end of exactly one minute draw off 2 c.c. of the solution and add it to the iodine in one of the tubes, and repeat this each minute. If the tubes are kept in the order

in which the additions are made, the colours will depend on the amount of action that has taken place. He states that if the malt extract be of the best quality, the first tube will be of a blue colour, the second red and the third or fourth yellow. This is a somewhat empirical method, and can give no quantitative valuation.

Estimation of Dextrin.—To 20 c.c. of a 5 per cent solution of the extract, add 250 c.c. of methylated spirit. If the precipitate be very small, a fresh experiment should be commenced with a 10 per cent solution. Determine the nitrogen in the washed and dried precipitate, and deduct the weight of the calculated proteids ($N \times 6.25$), returning the remainder as dextrin. It is more accurate to redissolve in water and precipitate a second time with alcohol.

Estimation of Proteids.—These are determined by a direct determination of nitrogen on 1 grm. of the extract by Kjeldahl's process. The total nitrogen varies from 0.5 to 2.2 per cent.

Rotatory Power.—The specific rotatory power of genuine malt extracts usually varies between $+80^\circ$ and 90° .

The following figures are those of a number of commercial malt extracts, No. 1 being one of the best-known brands and of the best quality.

	Solids.	Sp. gravity.	Sp. Rotation.	Reducing Sugars as Maltose.	Lintner Value.
				Per cent	
1	73	1.400	$+76^\circ$	62	38
2	74	1.390	$+80^\circ$	60	22
3	72.5	1.375	$+80^\circ$	57	37
4	73	1.380	$+82^\circ$	55	24
5	72	1.378	$+79^\circ$	58	14
6	71	1.378	$+78^\circ$	56	25
7	76	1.400	$+84^\circ$	48	—
8	78	1.420	$+88^\circ$	61	—
9	78	1.415	$+88^\circ$	53	—

Any sample with a rotation of over $+90^\circ$ is suspicious, and may be suspected to contain glucose syrup—the dextrin present in this causing it to have an average specific rotation of over $+100^\circ$.

THE STARCHES AND STARCHY FOODS.

The second group of the carbohydrates which comes within the scope of this work, is that embracing the starches and starchy foods. The microscopic characters of starches are the only means of identifying them with certainty, and their examination is principally microscopic rather than chemical.

Starch has the empirical formula $(C_6H_{10}O_5)_n$, its structural formula being unknown. It is a white tasteless powder not soluble in any solvent without some alteration. It is a cellular substance consisting of small masses of starch "granulose," encased in an outer layer of starch cellulose. When heated with water the cellulose layer is ruptured and the granulose and similar matters are dissolved in the

water. The characteristic blue colour which is given by starch when treated with iodine is due to the granulose. Starch solutions are highly dextrorotatory $[\alpha]_d$ = about $+200^\circ$. Starch is readily converted into dextrin and maltose by boiling with dilute acids, dextrose eventually resulting if the treatment be prolonged. A similar change is produced (on soluble starch) by malt extract, on account of the diastase present.

The Microscopic Examination of Starches.—Numerous investigations have shown that the starch grains produced by any particular plant are remarkably constant in size, shape and other characteristics. A small quantity of the starch is mixed on a slide with a few drops of water. In many cases a micrometer scale on the eyepiece is useful, as the size of the grains is sometimes an important characteristic to consider. Glycerine is not a good medium to mount starch in, since its high refractive power renders the striations in the starch less visible. When it is necessary to locate starch grains in a section, or in a mixed powder, the specimen should be irrigated with iodine solution, when the starch grains are stained from a violet to an almost black colour. When the starch grains are very small, as in cayenne pepper, such staining becomes necessary.

The following points are to be noted:—

(1) The shape, whether oval, ovate, ellipsoidal, oyster-shaped, nearly round, or even angular. To get a proper idea of the shape of a starch grain, a drop or two of alcohol should be brought to the edge of the cover glass, by which means a current is set up and the starch grains move and can be examined in motion. Some grains may be found adherent to each other, forming compound grains.

(2) The size of starch grains is usually expressed in microns (a micron, μ , is $\frac{1}{1000}$ part of a millimetre). The size varies from 5 microns to 70 microns in their longest measurement.

(3) The hilum. Near to one extremity, generally the narrower end, there is a point round which concentric striations are arranged; with a high focus it appears as a dark spot, and with a low focus as a light spot. In some grains there is a V-shaped fissure through the hilum, as this spot is called. The hilum is sometimes in the centre of the grain, sometimes eccentric. The ratio of the distance of the hilum from the nearer margin to that from the further margin is the measure of its eccentricity.

(4) Striations. Round the hilum there are a number of striations or striæ, concentric lines probably due to varying proportions of water in the different parts of the grain. With a high focus the striæ are dark, and light at low focus. Some starches exhibit no striations at all, whilst in others they are well marked.

It will here be convenient to describe the microscopic characters of the principal starches, many of which are illustrated on pp. 168-172.

Wheat Starch.—This starch is obtained from various species of *Triticum*. It consists of large nearly round and oval grains; with numerous very small ones, but with few of intermediate size. There is no evident hilum, nor striations in most of the grains, but by careful alteration of the focus, a very few will be found to show a distinct hilum in

the shape of a minute spot, a cleft or a small cavity. Rarely a faint concentric striation will be observed. On observing the moving grains, they will be found to be lenticular, and not spherical, and on side view a longitudinal line may often be observed. The size of the larger grains when lying flat varies from 20 to 35 μ .

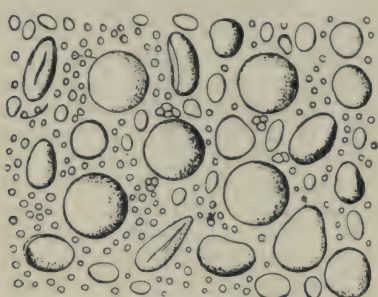


FIG. 10.—Wheat starch $\times 240$.

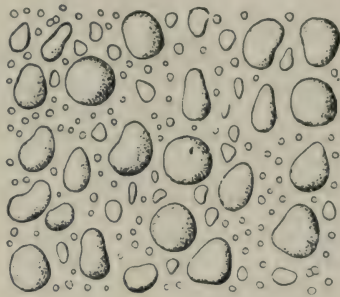


FIG. 11.—Barley starch $\times 240$.

(The illustrations of starches by Greenish & Collin are reproduced by permission of the Editor of the *Pharmaceutical Journal*).

Barley Starch.—This is obtained from *Hordeum distichon*. This starch is very similar to that of wheat, consisting of large and small grains with but few of intermediate size. The large grains are rather smaller than those of wheat starch, measuring from 15 to 25 μ , rarely up to 30 μ . They are also less regular in shape, the rounded grains often being of a somewhat kidney shape. On moving they are seen to be lemon shaped rather than lenticular. Hilum and striations are very rarely found.

Rye Starch.—This is obtained from *Secale cereale*. The grains closely resemble those of wheat starch, but the larger ones measure 40 to 50 μ , and are not so regular in shape. The grains frequently

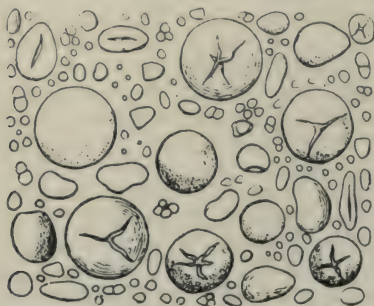


FIG. 12.—Rye starch $\times 240$.
(Greenish & Collin.)

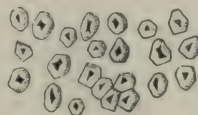


FIG. 13.—Maize starch $\times 240$.
(Greenish & Collin.)

show a fissure with 3 to 5 rays extending from an invisible hilum nearly to the circumference. No striations are visible.

Maize Starch.—This starch is obtained from *Zea mays*.¹⁰ The

grains are circular or polyhedral, usually with more or less rounded angles, measuring about 7 to 18 μ . They are nearly uniform in size, generally about 12 to 15 μ , and exhibit a well-marked hilum, which is sometimes a point, but usually a well-defined 3- or 4- rayed cleft. No striations are visible. Compound grains do not occur.

Oat Starch.—This is contained in the fruits of *Avena sativa*. The grains are nearly uniform in size, about 10 μ , but large grains up to 45 μ are plentiful, but are seen to be merely compound grains which are easily separated. The grains are mostly angular, sometimes round or lemon shaped and resemble rice starch in appearance. No hilum nor striations are to be found.

Rice Starch.—This is obtained from *Oryza sativa*. The grains measure from 6 to 10 μ , and many larger compound grains are present. Many are polygonal, usually 5- or 6-sided, rarely triangular, and no lemon-shaped nor round grains are present. No hilum is seen (except rarely when a small light central spot may be seen) and there are no striæ to be found.

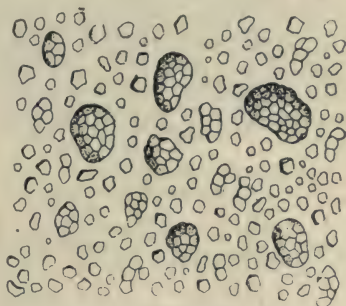


FIG. 14.—Rice starch $\times 240$.
(Greenish & Collin.)

Potato Starch.—This starch is obtained from the tubers of *Solanum tuberosum*. The size of the grains is very variable, the larger ones measuring up to 100 μ or even 120 μ in length, whilst the small and medium-sized grains measure from 15 to 65 μ . The grains



FIG. 15.—Potato starch $\times 240$. (Greenish & Collin.)

are flattened, and are oval, ellipsoidal or, especially in the case of the larger grains, oyster-shaped. The hilum is a spot nearly always close

to the smaller end of the grain, and the striations are concentric and exceedingly well marked. A few compound grains are to be found.

Arrowroot Starch.—This is obtained from various species of



FIG. 16.—Arrowroot starch $\times 240$.
(Greenish & Collin.)

Maranta (West Indies, Natal, Bermuda, or St. Vincent). The grains are fairly large, measuring from 10 to 70 μ in length. They somewhat resemble potato starch, but are smaller and less regular in shape; they are ovoid, and often shaped something like a mussel shell or a pear, and sometimes tend towards a triangular shape. The small grains are nearly spherical. The hilum is well marked,

and is usually near the narrow end of the grain. It is either circular or linear and often cracked, so that the cleft appears like the open wings of a bird. In St. Vincent arrowroot, the linear or stellate hilum predominates, whilst in the Natal variety, the rounded hilum is more usual. Striations are numerous and well defined, but not very strongly marked. *Tous les mois* arrowroot is obtained from *Canna* species—the grains are similar to normal arrowroot starch, but are larger (50 to 150 μ) with a rounded hilum and well marked striations. Curcuma or East Indian arrowroot is also similar; the grains are 30 to 60 μ in length. The grains taper to a small obtuse projection in which the hilum

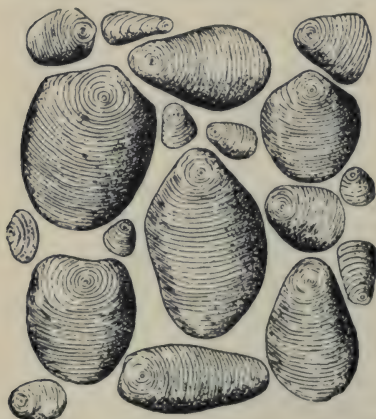


FIG. 17.—Tous les mois starch $\times 240$.
(Greenish & Collin.)

is situated. The concentric striations are well defined, but not very strongly marked.

Sago Starch.—This is prepared from the sago palm, *Metroxylon Sagu*. The grains vary in length from 25 to 65 μ . Pearl sago is prepared from sago starch with the aid of heat, so that in commercial sago that has thus been agglomerated, the granules are mostly broken. The intact starch grains are ovoid, often rounded at the larger end and truncated at the narrower end. Many are very irregular in shape,

some are simple, but many are compound, having one or more small granules attached to short protuberances on a large grain. The hilum is eccentric, and is a circular spot or crack at the broader end of the grain. Striations are concentric, often plain, but frequently only faintly indicated.

Tapioca Starch.—Tapioca is prepared in a similar manner to prepared sago, but from the starch obtained from the tubers of *manihot utilissima*. The grains of the manihot starch are from 15 to 35 μ in length, and are therefore small. Many of them exhibit a flat surface in places, having probably been components of a compound grain. They are circular or kettle-drum in shape, many having the flat surface forming a sharp angle when they meet. The hilum is a point or short cleft, and is nearly central, but this depends on the point of observation. If the grain be lying flat the hilum appears eccentric—if standing on a flat surface, it will appear central. In commercial tapioca, most of the grains exhibit the effects of heat.

Bean Starch.—This is prepared from *Phaseolus vulgaris*, the haricot bean. The grains vary in size from 25 to 60 μ , most of them measuring about 35 μ in diameter. They are oval, or reniform in shape, some being nearly round. The hilum, owing to large fissures, appears either stellate, or as a long and often branching cleft running nearly the whole length of the grain, and appearing very conspicuously under the microscope. The striæ are well marked.

Banana Starch.—This starch is prepared from the unripe fruits



FIG. 18.—Sago starch $\times 240$.
(Greenish & Collin.)



FIG. 19.—Bean starch.

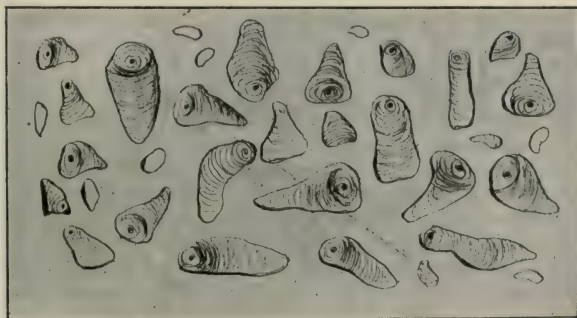


FIG. 20.—Banana starch.

of *Musa sapientum*; the grains are oval, ellipsoidal, or elongated. The hilum is rounded near to the extremity and surrounded by concentric striations. Size from 7 to 65 μ .

Pea Starch.—This occurs in the seeds of *Pisum sativum*. The grains are very similar to those of bean starch, but are rather smaller, measuring about 20 to 40 μ . So similar is this to bean starch that in a mixture of the two, it could scarcely be detected.



FIG. 21.—Pea starch.

With polarized light, starch grains show dark crosses, the point of intersection of the arms being at the hilum. Some starches show colours with crossed prisms and a selenite plate, but in the author's experience, the value of the polarization phenomena with starch has been much exaggerated, and no information is yielded that is not easily obtainable by the use of ordinary light.

Passing on from the separated starches to the flours of the principal starchy foods, the following are the principal diagnostic characters.

Wheat Flour.—Microscopic characters. The characteristic features are:—

1. The large starch grains.
2. The hairs with enlarged lumen at base.
3. The thick-walled pitted cells of the hypoderma.
4. The tabular cells of the outer epidermis with pitted transverse walls.

Rye Flour.—The characteristic features are:—

1. The starch grains which are rather larger than those of wheat and often show a stellate hilum.
2. The hairs with thinner walls than those of wheat, and less enlarged lumen.
3. The lignified cells of the hypoderma which are usually longer than the transverse cells, whereas in wheat they are shorter.

Barley Flour.—The characteristic features are:—

1. The typical starch grains.
2. Epidermal cells of the paleæ with thickened sinuous walls.
3. Hairs on the inner epidermis of the paleæ.
4. The thin-walled, not pitted, epidermal cells of the pericarp.
5. The aleurone grains of two or three rows of cells.

Oat Flour is distinguished by:—

1. The elongated hairs which are often found in pairs.
2. The cells of the outer epidermis of the pericarp which have thin walls and numerous pits.
3. The polygonal cell of the hypoderma.
4. The cells of the seed coat which are polygonal, smooth, and seldom pitted.

5. The starch grains, mostly compound, and composed of small angular grains.

Rice Flour contains only a small proportion of the seed coats consisting chiefly of the characteristic starch grains, compound and small simple, rounded or polyhedral grains.

Maize Flour.—The characteristic features are :—

1. The typical starch grains.
2. The numerous small tabular grains of the pericarp.
3. The hypodermal cells with slightly pitted walls.

Buckwheat Flour.—The characteristic features are :—

1. The typical starch grains which resemble rice starch but are rather more rounded, with a small central hilum, and usually agglomerated.

2. The epidermal cells of the seed coat which have sinuous walls.

3. The middle layer of the seed coat which consists of cells with lacunæ.

Haricot Bean Flour.—The characteristic features are :—

1. The typical starch grains which are ovoid with an elongated or fissured hilum.

2. The palisade cells of the epidermis.

3. The rectangular cells containing prismatic crystal of calcium oxalate.

4. The cells of the cotyledons which are polygonal and thickened at the angles.

Pea Flour.—The characteristic features are :—

1. The palisade cell with square ends.

2. The starch grains which bear rounded protuberances, and have a central hilum surrounded by concentric rings.

Lentil Flour.—The characteristic features are :—

1. Palisade cells with conical ends.

2. Hour-glass cells without calcium oxalate.

3. Starch grains intermediate in character between pea starch and bean starch, the hilum being generally fissured with distinct striæ.

Wheat Flour and Bread.—Wheat flour is understood to be the ground fruit of *Triticum sativum* and allied species (freed from the bran or episperm).

The average composition of wheat flour, according to various chemists, is as follows :—

	Graham.	Church.	Konig.	Bell.
	Per cent	Per cent	Per cent	Per cent
Water	11.1	13 to 14.5	13.56	12.08 to 14.08
Starch	62.3	69 „ 74	64.07	63.71 „ 65.86
Fat	1.2	0.8 „ 1.2	1.70	1.48 „ 1.56
Cellulose	8.3	0.7 „ 2.6	2.62	2.93 „ 3.03
Sugar and gum	3.8	—	3.82	2.24 „ 2.57
Albumenoids	10.9	10.5 „ 11.0	12.42	11.59 „ 15.53
Mineral matter	1.6	0.7 „ 1.7	1.79	1.60 „ 1.74

If the whole of the grain be ground, in order to prepare whole-meal flour, the percentage of mineral matter is considerably raised, bran containing as much as 7 per cent of ash.

The fat of wheat consists of olein and palmitin with a small amount of similar glycerides, and about 6 per cent of free fatty acids.

Small quantities of dextrin are present, but it is never necessary in practice to determine this.

The proteids of wheat are several in number and are generally mentioned here as "albumenoids". The amount of nitrogen present in the greater part of the proteids and allied bodies of the cereals contains an amount of nitrogen not differing much from 15·8. This value is taken as a fair average one, so that the total nitrogen multiplied by 6·33 is usually returned as total albumenoids (5·7 gives more accurate results—in any case the multiplier should be stated). A more complete examination of the nitrogenous bodies of wheat flour is, however, often necessary. According to Osborne and Voorhees ("American Chem. Journal," xv. 392; xvi. 524), wheat contains five different proteids which have the following composition:—

Properties.		Composition.				
		C.	H.	N.	O.	S.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Globulin	0·6 to 0·7	51·03	6·85	18·39	23·04	0·69
Albumin	0·3 „ 0·4	53·02	6·84	16·80	22·06	1·28
Proteose	0·3	51·86	6·82	17·32	24·00	
Gladiin	4·25	52·72	6·86	17·66	21·62	1·14
Glutenin	4 to 4·5	52·34	6·83	17·49	22·26	1·08

The two last named are the principal constituents of the gluten, the first three being soluble in water and only present in small amount. They are separated in the following manner:—

Four thousand grms. of flour are treated with 8000 c.c. of 10 per cent brine, allowed to stand all night, and the supernatant liquid filtered off. Another 2000 c.c. of brine are added to the residue, the whole stirred up, allowed to settle, and again filtered. The filtrate is at once saturated with ammonium sulphate, and the precipitated globulin filtered off, redissolved in 10 per cent brine, and dialysed until all the salts are removed when practically pure globulin is precipitated. The albumin is coagulated by heating the globulin-free solution and proteose is precipitated by saturating the filtrate with sodium chloride.

Glutenin is prepared by boiling crude gluten (made by kneading flour in a stream of water, so that the starch and soluble proteids are washed away) with alcohol of specific gravity 0·890. The gliadin dissolves, leaving a residue of glutenin with a small quantity of impurities. It can be purified by dissolving in very dilute potash and

neutralizing with acetic acid, when it is precipitated as a whitish-grey mass. It is practically insoluble in cold water and cold alcohol.

Gliadin is readily dissolved from the flour or the gluten by hot dilute alcohol. It is easily soluble in dilute alcohol and in water free from salts, and forms, in the hydrated condition, a sticky mass, which, however, when dehydrated by treatment with absolute alcohol, and then treated with ether, forms a white friable mass easily powdered.

Wheat-gluten, then, consists essentially of a mixture of gliadin and glutenin. Gluten cannot properly be made with distilled water, since gliadin is easily soluble unless salts be present. It has been suggested that the gluten is the result of a ferment action on the flour in the presence of water. Osborne and Voorhees, however, state that "no ferment action occurs in the formation of gluten". No cereal but wheat yields a gluten, which has considerable importance as a food stuff. Bread made from gluten has long been recommended as a suitable food for diabetic patients. Many preparations, however, contain so much starch left in the gluten as to be totally unfit for this purpose, and in addition such bread is very unpalatable. Gluten bread should be examined for starch, and if present in more than traces, this should be determined. It has now, however, been nearly superseded by a bread made from the casein extracted from milk, which is obtainable practically free from carbohydrates.

In the examination of flour (from the point of view of the baker, technical and empirical valuations of the flour are often required which are not within the scope of this work—see "Bread-making"—by W. Jago), the following determinations are more or less necessary:—

(1) Water, (2) mineral matter, (3) fat, (4) cold water extract, (5) total nitrogen, etc., (6) starch, (7) percentage of gluten obtainable, (8) amount of free acidity, (9) fibre.

A microscopic examination, bearing in mind the diagnostic features above given, especially when a comparison is made with a standard sample, is, of course, essential.

Determination of Water and Mineral Matter.—Water should be determined on about 2 grms. of the flour, at 105°, and the dried flour may then be ignited for the ash determination. The water averages about 12 to 14 per cent and the mineral matter from 0.5 to 0.7 per cent. The ash consists principally (80 per cent) of potassium phosphate.

Determination of Fat.—The flour must be dried before the fat is determined, due allowance being made for the loss in weight. From 10 to 15 grms. should be exhausted with dry ether in a Soxhlet tube in the usual manner. The fat will be found to be from 1 per cent to 1.6 per cent.

Cold Water Extract.—The estimation and examination of the cold water extract afford much useful information. Ten grms. of the flour are rubbed to a cream with water and the volume made up to 500 c.c. and the whole well shaken at intervals from two to three hours. The liquid is filtered, and aliquot parts used for the following determination. A portion—about 200 c.c.—is evaporated and the residue

weighed. This, the cold water extract, should vary from 3 per cent to 7 per cent, usually from 5 per cent to 6 per cent. To some extent the cold water extract may be regarded as a measure of the amount of degradation of the starch of the wheat, an excess of soluble matter indicating unsoundness in the flour. If desired the soluble proteids may be estimated by evaporating down 200 c.c. of the extract to a syrupy consistence and determining the nitrogen present by Kjeldahl's process. The soluble proteids (using the nitrogen factor 6.33) will vary from 0.7 per cent to 1.5 per cent, occasionally being a little lower or a little higher than these limits. If the sugar is required, it is estimated with sufficient accuracy by inverting a portion of the aqueous extract and determining its reducing power. The amount calculated as sucrose is from 2.5 per cent to 3 per cent. It is probable that in perfectly fresh sound wheat sucrose is the only sugar present, but that if any diastatic action has set in, a little maltose may be present. This is best decided by extracting the flour with 70 per cent alcohol, and determining the sugar before and after inversion in the alcoholic extract. The presence of much maltose would indicate that the flour was not a fresh, sound one.

Determination of Total Proteids.—This is effected by a nitrogen determination on 1 gm. of the flour, rubbed into a cream with water so as to prevent the formation of lumps. As pointed out above, the nitrogen factor 6.25 is usually used in calculating the proteids, but 5.7 is more correct. The multiplication used should, therefore, be stated.

Determination of Starch.—O'Sullivan's process ("Journal Chem. Soc." xlv. 1) is the most accurate, and is easily carried out. Five grms. of the flour are placed in a flask holding about 120 c.c., and which has been well wetted with alcohol; 25 c.c. of ether are then added. The closed flask is well shaken from time to time, and after a few hours the ether is filtered off and the residue washed several times with more ether. To the residue 80 c.c. of alcohol of specific gravity 0.9 are added and the mixture kept at 35° C. and shaken from time to time, for several hours. The alcoholic solution is then filtered through the same filter that was used to filter off the ether, and the residue well washed with more alcohol at the same temperature and of the same strength. The residue in the flask and on the filter is then treated with about 500 c.c. of cold water. After twenty-four hours the clear supernatant liquid is carefully decanted through a filter and the residue repeatedly washed with water at 38° C. The residue is then transferred to a 100 c.c. beaker, the solid matter and the water used to aid its transference to the beaker, measuring about 40 to 50 c.c. This is heated to 100° for a few minutes with constant stirring to effect the gelatinization of the starch. When the contents of the beaker are cooled down to 60°, about 0.03 gm. of diastase, dissolved in a few c.c. of water, is added. After a short time, conversion into maltose and dextrin is complete. The reaction is complete when a drop of the liquid does not give a blue colour with starch. But by keeping the mixture at about 62° for another hour, it filters far more readily. It is now heated to 100° for ten minutes and filtered, the

residue on the filter being well washed with hot water. On cooling the filtrate is made up to 100 c.c. and the specific gravity is taken. The maltose is estimated by reduction of Fehling's solution; the dextrin can be deduced by a polarimetric reading. The amount of maltose multiplied by 0.95 gives the corresponding amount of starch, which, added to the dextrin found, should amount to the solids deduced from the specific gravity of the solution (i.e. by dividing the excess of specific gravity over 1000 by 3.86). An example given by O'Sullivan will make the calculations clear.

In the analysis 4.94 grms. of the ground wheat were taken. The 100 c.c. solution had a rotation equivalent = 8.52° in a 200 mm. tube. By Fehling's method it was found to contain 2.196 grms. of maltose. But $2.196 \times 2.78^\circ = 6.10^\circ$, the rotation due to the maltose ($[\alpha]_d = +139^\circ$), therefore 2.42° is due to the dextrin ($[\alpha]_d = +200.4^\circ$), so that $\frac{2.42}{4.008} = 0.605$ grms. of dextrin per 100 c.c. Hence maltose, $2.196 \times 0.95 = 2.086$. This, added to 0.605 = 2.691, the total amount of starch present in the 4.94 grms. of wheat used.

If the acid conversion method be used, the gelatinized starch should be heated to 95° to 100° with 200 c.c. of water and 20 c.c. of strong hydrochloric acid for $2\frac{1}{2}$ hours under a reflux condenser. When cool the liquid is clarified, if necessary, with alumina cream, neutralized with alkali solution and made up to 500 c.c. after filtration. The dextrose is determined by reduction of Fehling's solution in the usual manner. Dextrose $\times 0.9$ may be taken as the equivalent of starch.

If diastase be not at hand, a fairly accurate result is obtained by replacing it by sulphuric acid and inverting in the usual manner. Wheat flour will usually contain over 60 per cent of starch.

Percentage of Gluten obtainable.—This determination is somewhat empirical, but is necessary in certain cases: 10 or 20 grams are rubbed in a mortar with 15 to 20 c.c. of water. The dough is then tied up in a fine muslin cloth, rather tightly, and gently kneaded with the fingers under a gentle stream of water until no more starch is washed out, as shown by the clearness of the water running away. The gluten is then taken out of the cloth and flattened in a dish and slowly dried at 110° to 115° C. It generally amounts to about 35 per cent.

Acidity of the Flour.—By rubbing the flour into a cream and then adding water, and titrating the whole, a higher acid value will be obtained than by titrating an aqueous extract. If the flour be so titrated it should not show a higher acid value than about 0.1 per cent calculated as sulphuric acid, H_2SO_4 . By extracting the flour with water and titrating directly the liquid is filtered 0.025 per cent is about the highest value a good flour will give.

Crude Fibre.—The amount of fibre may be determined as described on p. 21.

Adulterants of Flour.—Adulteration of flour with the flour or meal of other cereals can, practically, only be determined by a careful microscopic examination, and any suspected additions should be compared with a standard sample of the suspected adulterant. Maize flour, rye flour, rice meal, potato starch and leguminous starches have

been found, but the adulteration of wheat flour is not very prevalent.

Seeds, sometimes of an objectionable nature, occasionally get mixed with the wheat, and some of these can be detected by the microscope. The bearded darnel (*Lolium temulentum*) is one of these. The starch grains of this seed are somewhat similar to those of rice, but the principal cellular tissue of the husk in the darnel consists of cells only twice or thrice as long as they are broad, whereas in the rice they are long and narrow fibres. In the presence of much darnel an extract of flour with 90 per cent alcohol will be of a slight greenish tint, gradually darkening, and on evaporation of the alcohol an acrid greenish resin is left.

The seeds of the corn cockle (*Agrostemma githago*) are sometimes also mixed with the wheat. The surface of the seed coat is quite characteristic and shows very large thick-walled cells of up to half a millimetre in diameter, with branching protuberances on the surface. The starch grains, situated in the parenchymatous cells, are very minute, and characteristic egg-shaped grains consisting of starch, saponin, and other bodies are to be found.

Ergot in flour (which, although a natural impurity) is of sufficient importance to be looked for carefully in every case of obviously low grade, stale flour.

Vogel ("Chem. Central." 3, x. 559) recommends staining the flour with aniline violet. Starch granules which have been attacked by ergot or any other fungus, are stained an intense violet, the other grains being left nearly colourless. Petri ("Zeit. f. Anal. Chem." 1879, 211) recommends exhausting 20 grms. of the flour with hot alcohol. If ergot be present the extract will be more or less reddish in colour. If the alcoholic extract be diluted with twice its volume of water and shaken with ether, amyl alcohol, benzene or chloroform, each of these solvents will be coloured reddish to full red. Or if 20 drops of dilute sulphuric acid be added to the alcoholic solution, and the liquid examined spectroscopically, two well-defined absorption bands will be seen if the flour be ergotized, one in the green near E, and a wider band in the blue between F and G. According to Hoffmann and Hilger ("Archiv. der Pharm." 1885, 827) 0.01 per cent of ergot in flour can be detected by treating 10 grms. of flour with 10 drops of a 20 per cent solution of potash, and allowing it to stand for ten minutes. Thirty c.c. of ether and 20 drops of dilute sulphuric acid are then added, and the whole allowed to stand for six hours with occasional shaking, and then filtered. The residue is washed with ether so that 20 c.c. of filtrate are collected, 10 to 15 drops of a saturated solution of bicarbonate of sodium solution are then added and the whole well shaken. In the presence of ergot a well-marked violet colour is developed. This reaction is very reliable.

Vogel ("Verfälschungen des Mehls") gives the following chemical test for several adulterants: Two grms. of the flour are warmed and shaken with 10 c.c. of 70 per cent alcohol to which 5 per cent of hydrochloric acid has been added. The liquid is filtered. Wheat and rye flour give a practically colourless extract; if 5 per cent of corn-

cockle be present, the extract will be of an orange colour; barley or oats give a yellow colour; leguminous flours give a reddish colour if from 5 per cent to 10 per cent be present, or a violet colour if more be present; in the presence of 5 per cent of ergotized wheat, the extract will be of a blood-red colour. Numerous "methods" for empirically detecting more or less apocryphal adulterants have been published from time to time, but as most of them are quite useless, they are not referred to here.

Embrey points out ("Analyst," xxv. 315) that foreign so-called wheaten flour, and certain flours sold under fancy names such as "self-raising flour," etc., often contain from 10 per cent to 20 per cent of maize flour.

He considers that an examination of the ash is of some value, stating that the ash of maize starch contains 38.65 per cent of phosphates calculated as tricalcium phosphate, whilst that of wheat flour contains only 22.42 per cent. These figures, however, are quite at variance with those of other observers, and are not, in the author's opinion, of the slightest value.

Bauman states that as little as 2 per cent of maize in wheat flour can be detected in the following manner: About 0.1 grm. of the flour is mixed with 10 c.c. of a 1.8 per cent solution of potassium hydroxide and the test tube shaken at intervals during two minutes. Four or five drops of 25 per cent solution HCl are then added and the tube is again shaken. The liquid must remain alkaline. If a drop of the liquid be now examined microscopically the wheat starch will be completely ruptured, whilst the maize starch will be unaltered. It is suggested that an approximate determination can be made by the comparison of the sample with standard mixtures under identical conditions.

A. C. Wilson states that maize starch may be detected by examining the flour in a clove oil preparation. The hilum of maize starch appears as a black dot or star, whilst that of wheat flour is invisible. This is confirmed by E. J. Bevan ("Analyst," xxv. 316).

Mineral Adulterations.—The principal mineral adulterant to guard against is alum, which is added in very small quantity in order to whiten the flour. Any other mineral matter, such as plaster of paris, is very rarely found. If such be suspected it will usually show in the ash, as any figure over 0.75 per cent should be suspected. Mineral matter may be detected by the chloroform test—it being remembered that as a rule there is only a small amount added, if any, not to increase the weight, but to conceal the bad colour of an inferior flour. One hundred grms. of the flour are shaken in a separator with 250 c.c. of chloroform and allowed to settle; the flour will be on the surface, and the mineral matter will have settled at the bottom of the separator. This is run off and diluted with a little more chloroform, and again run off and the chloroform evaporated in a current of air. The deposit may be examined microscopically to detect crystals of alum, and then extracted with water, in which the alum if present may be determined as Al_2O_3 : if the insoluble residue does not weigh more than 0.1 per cent of the weight of the flour it need not be further examined.

Alum may be detected in flour by the following methods. Ten grms. of the flour are rubbed down with 10 c.c. of water and 1 c.c. of a tincture of logwood (5 per cent logwood in alcohol) and 1 c.c. of a saturated solution of ammonium carbonate are then added and the whole well mixed. If the flour be pure, a pink colour gradually changing to a dirty brown results: if alum be present, the colour changes to a lavender or blue colour; or strips of gelatine may be soaked for twelve hours in the mixture of water and flour and then immersed in the alkaline solution of logwood, when a decided blue colour is obtained on the gelatine.

If bread has to be dealt with instead of flour, the following points are to be noted. The following represent the average composition of moderately fresh bread prepared from wheaten flour:—

	Per cent
Water	28 to 45
Albumenoids	5 „ 7
Fat	0.4 „ 1
Sugar	0.8 „ 4.5
Starch (and dextrin, etc.)	38 „ 58
Cellulose	0.2 „ 0.8
Mineral matter	0.75 „ 1.4

From the analyst's point of view, the examination of bread in practice is usually restricted to the detection and determination of alum. Cases of adulteration with other starchy matter are rare, and as the starch grains are much altered by the action of heat, a microscopic examination will be of little service, unless comparisons are made with bakings from the flours suspected to have been used. A little salt, or the reaction products of baking powders, are to be found frequently, but no exception is to be taken to these. It is said that sulphate of barium and plaster of paris are sometimes added, but if so, this is very rare, and such additions will be found in the ash of the bread, which will, of course, be correspondingly high.

The principal adulterant of bread, however, is alum, which is added to cover the use of inferior flours.

Alum may be detected in bread by diluting 5 c.c. of the tincture of logwood mentioned under flour, with 90 c.c. of water and 5 c.c. of saturated solution of ammonium carbonate. The liquid is then poured on about 10 grms. of the bread on a glass dish. After about five minutes the liquid is drained away and the bread washed gently with a little water and dried. If alum be present, the bread will assume a lavender or dark blue colour when dry. According to Allen, 7 grains of alum in a 4 lb. loaf can thus be detected. Young, however, considers that this test is not absolutely reliable, as some breads free from alum give the reaction.

For the quantitative determination of alum Dupre's process, slightly modified by Young ("Analyst," xv. 61, 83) gives the most accurate results. One hundred grms. of bread are incinerated in a muffle, until the ash does not weigh more than 2 grms. This is then moistened with 3 c.c. of hydrochloric acid and 25 c.c. of distilled water. The whole is boiled, filtered, and the undissolved matter is washed, dried, ignited,

and weighed. This consists of silica. Ammonia solution (dilute) is then added until the precipitate formed barely dissolves. The liquid is then raised to boiling-point and a faintly acid solution of ammonium acetate added, and the boiling continued for a few minutes. The precipitate of iron and aluminium phosphates should be filtered off at once. (If this is done in the cold or after standing for long the results are much below the truth.) The precipitate is then washed, and redissolved in a very small quantity of dilute hydrochloric acid. The resulting solution is poured into an excess of a solution of *pure* caustic soda, and after heating for a short time, the liquid is diluted and filtered. The filtrate is acidified with slight excess of HCl and ammonium acetate and a few drops of solution of sodium phosphate are added, and finally a slight excess of ammonia. The liquid is heated till all the ammonia has been driven off, and the precipitated aluminium phosphate is filtered off, washed, dried, ignited, and weighed. The weight of the precipitate multiplied by 3.87 gives the equivalent of crystallized alum, but this requires a correction, as bread naturally contains traces of aluminous silicates which have been derived from the mill-stones, or from the soil, and the alumina thus naturally present must be deducted.

From a series of forty analyses by Carter Bell, it is clear that the silica and alumina are combined in such proportions that the correction should be to allow for any part of silica found an equal amount of crystallized alum, which is deducted from the result above found.

Bleached Flours.—Nitrous fumes are sometimes used for bleaching flours, in order to apparently improve their quality. It has been shown that this seriously affects the ease with which certain constituents of the flour are digested, and also causes the bread baked from such flour to have a greater tendency to go mouldy. Such bleached flours may be recognized by determining the presence of nitrites, and also by determining the iodine value of the fat extracted from the flour. Normal flours yield a fat with an iodine absorption of 100 or over, whereas bleached flours give a fat with an iodine value of from 80 to 90, owing to oxidation of the fat.

Nitrites may be detected by the Griess-Ilosvay's reaction. If a bleached flour be treated with a solution of naphthylamine acetate and sulphanilic acid, an amino-azo dye of a red colour is at once produced. An unbleached flour will show no coloration for at least half an hour. Occasionally an unbleached flour appears to answer this reaction however. Weil ("Chem. Zeit." 1909, **33**, 29) states that it is well known that bleached flours revert in colour fairly rapidly if stored. This may be used to detect the bleaching, if the reversion be accelerated. This is best done by passing a current of sulphuretted hydrogen through the flour in a closed vessel for an hour. An unbleached flour shows hardly any change whereas the colour of a bleached flour is much darker than it was before such treatment.

Maize Flour.—The flour of maize or Indian corn, used largely under the name of corn flour.

The microscopic examination of this flour will yield most of the information that is necessary. Rice or potato flours are the most pro-

bable adulterants and those should be specially looked for under the microscope.

The average composition of maize flours is :—

	Per cent
Fat	3.58
Starch	64.66
Cellulose	1.86
Sugar	1.94
Albumenoids	9.67
Other nitrogenous matter	4.60
Mineral matter	1.85
Moisture	12.34

The oil extracted from the flour may be examined. It should have the following character :—

Specific gravity	0.921 to 0.925
Iodine value	116 „ 123
Refractive index at 15°	1.4768 „ 1.4775
Saponification value	188 „ 189

Oatmeal.—This meal has the average composition :—

	Per cent
Water	10.07
Albumenoids	14.66
Fat	5.91
Sugar	2.26
Dextrin or gum	3.08
Starch	59.39
Mineral matter	2.24
Cellulose	2.89

Its characteristics are its high nitrogen value, and its high amount of cellulose tissues. Its fat content is also higher than most cereal grains and should be determined.

The chief adulterant of oatmeal is barley meal. This is detected by a microscopical examination. To determine the approximate amount of such adulteration, standard mixtures must be made of the two meals and a number of microscopic preparations made. The relative number of starch grains of the two meals is counted over a number of fields and the average taken. This is compared with standard preparations say of 20 per cent adulteration, 30 per cent, 40 per cent, etc., and so an approximate determination is arrived at.

Rice.—The only practical question arising for the analyst in regard to rice, is that of “facing”. Some rice millers use various substances to improve the appearance of rice grains, especially on the Continent. The Local Government Board published a report by J. A. Hanwill on the whole question (“Reports of Inspectors of Foods,” 1909, No. 8, 1-21). It is pointed out that the operation of milling rice includes the polishing of the grains, which is carried out in revolving cylinders lined with sheepskin. Some millers, however, add talc to improve the polish of the grain. Traces of ultramarine or aniline blues are also added to improve the colour, and a trace of oil to add translucency.

A glazing mixture—talc, glucose, glycerine and starch—is also sometimes used after the polishing process. From the results of analysis

published in the above report, it may be seen that the amount of such added substance is infinitesimal so far as the polishing is concerned, but that up to 0.2 per cent of mineral matter may be added by the glazing process. Fourteen samples of rice milled in Holland contained from 0.16 to 1.25 per cent of added mineral matter (a normal rice contains from 0.2 to 0.3 per cent of mineral matter). The conclusion arrived at by the reporter is that on the whole it seems regrettable that the practice of polishing rice with mineral matter has been allowed to reach its present proportions, but as the matter has become a trade custom, it is suggested that an outside of limit of 0.5 per cent of mineral matter might be fixed for rice.

Borgherio ("Giorn. Farm. Chim." 1909, **58**, 533) states that he has found samples of rice dyed a faint yellow colour with an oil-soluble aniline yellow. This is done to improve the appearance of poor samples, and may be detected by heating the ethereal extract of the rice with hydrochloric acid. The acid layer becomes a faint carmine pink if this dye is present, changing to yellow on the addition of ammonia.

Richardson ("Analyst," xxxv. 293) determines the extraneous mineral matter in rice in the following manner. Five grms. of the rice grains are treated in a platinum dish with 0.5 gm. of ammonium fluoride, 2 c.c. of water and 2 c.c. of strong hydrochloric acid, and stirred occasionally during ten minutes with a platinum wire. The rice is then well washed with water, which is decanted off, and the so cleaned rice is incinerated.

The difference between the ash so found and the total ash (both recarbonated) gives the amount of facing mineral matter. Richardson finds the average natural ash of rice to be 0.2 per cent so that any excess over this may be regarded as added mineral matter.

Semolina and Macaroni.—Semolina is the coarse meal ground from certain varieties of hard or "durum" wheats now grown in France and certain parts of the United States and Canada, though originally produced in Italy, Sicily, and Russia. The hard wheats contain a considerable amount of gluten, and are therefore particularly suitable for the preparation of macaroni and the various pastes. In preparing the wheat, the husk is removed by wetting, heating, grinding, and sifting. The meal thus obtained—viz. semolina—is in small, round glazed granules.

Italian Pastes.—Semolina is the chief constituent of the Italian edible pastes. It is mixed with warm water, kneaded and moulded into different shapes by pressure through holes in an iron plate or by other similar methods, then dried. It has been said that the juices of carrots, onions, and other vegetables are mixed with the paste in some parts of Italy, but this is only used locally. Saffron is sometimes added to pastes for flavouring purposes, though sometimes in such small quantities that it would appear as if it were solely used to give a colour resembling egg-paste.

Macaroni is the larger slender tube or pipe-shaped variety; vermicelli is the worm-shaped product obtained when the holes in the plate are very small; spaghetti is a cord-like variety of a size between the two already mentioned.

Other kinds of Italian pastes or pâtes are prepared by rolling the kneaded semolina into thin sheets then cutting it into various shapes—as animals, letters of the alphabet, etc.

The following table shows the composition of some of these products :—

	No. of Samples.	Water.	Protein.	Fat.	Total Carbo-hydrates.	Crude Fibre.	Ash.
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Semolina	—	10.50	11.96	0.60	75.79	0.50	0.65
Macaroni	11	10.3	13.4	0.9	74.1	—	1.3
Noodles	2	10.7	11.7	1.0	75.6	0.4	1.0
Spaghetti	3	10.6	12.1	0.4	76.3	0.4	0.6
Vermicelli	15	11.0	10.9	2.0	72.0	—	4.1

Adulteration of Pastes.—The cheaper forms of semolina have been adulterated with rice, corn and potato flours but not often in this country.

Analysis of Pastes.—Determination of lecithin-phosphoric acid—Juckenack's method. This determination will indicate the presence or absence of eggs in the pastes. Egg-free pastes will give a result of about 0.02 per cent, and each egg per lb. of paste will give an average increase of 0.028 per cent. Extract 30 grms. of the finely ground sample with absolute alcohol for ten hours in a Soxhlet tube at a temperature inside the Soxhlet not below 55° to 60° C. There should be a small quantity of pumice stone in the extraction flask to prevent bumping during boiling, and the Soxhlet should be enveloped in asbestos if it is difficult to keep up the required temperature. After the extraction add 5 c.c. of alcoholic solution of potash (made by dissolving 40 grms. of phosphorus-free caustic potash in 1000 c.c. alcohol), then distil off all the alcohol. Transfer the residue to a platinum dish by means of hot water, evaporate to dryness on a water bath, and char over asbestos. Add dilute nitric acid to the charred mass, filter and wash with water. Again transfer the residue with the paper to the platinum dish, and burn to a white ash; then treat with nitric acid, filter and wash as before, uniting the filtrates. Determine phosphoric acid by the usual method.

Detection of Artificial Colours in Paste.—Turmeric, saffron, annatto, naphthol yellow (Martius yellow), naphthol yellow S, picric acid, aurantia, victoria yellow, tartrazine, metanil yellow, azo yellow, gold yellow and quinoline yellow are the colours that have been used in several of these pastes. Naphthol yellow, picric acid, metanil yellow and victoria yellow are injurious to health, and it is, therefore, improper to use them in European countries and the United States. They are not often found in the products now on the market.

The natural colouring matter of the flour and the lutein of eggs make it difficult to detect artificial colours. Ether will extract the

former, though it does not remove the artificial colours, which, however, mostly dissolve freely in the solvent if unmixed.

Juckenack's Method.—Take two portions of the sample each of about 10 grms. in test tubes with 15 c.c. of ether, and 15 c.c. of 70 per cent alcohol respectively; allow to stand for twelve hours.

(a) If the ether remains colourless, or only slightly tinted, and the substance below remains yellow while the alcohol is distinctly coloured and the substance below is decolorized, then a foreign dye is present.

(b) If both ether and alcohol are coloured either (1) lutein (egg colour) alone, or (2) this with a foreign dye is indicated.

(1) Add dilute nitrous acid to a portion of ether solution. If the ether is not completely decolorized a foreign dye is present.

(2) If the substance below the alcohol is decolorized, while that below the ether is coloured, the following tests for foreign dyes should be applied. Shake the portion previously treated with ether with three or more fresh portions of the same solvent until no more colour can be extracted, then shake the residue with 70 per cent alcohol and allow it to stand for twelve hours. Filter, then concentrate the solution, slightly acidify with hydrochloric acid, boil with wool which will be coloured if coal-tar dyes be present.

Schlegel's Method.—Extract 100 grms. of the finely powdered sample with ether in a continuous extraction apparatus. Shake the residue with a mixture of 140 c.c. of alcohol, 5 c.c. of ammonia and 105 c.c. of water at frequent intervals for half a day. Filter, evaporate to remove alcohol and ammonia, slightly acidify with hydrochloric acid, then filter again. Boil the filtrate with fat-free wool and identify the colour on the dyed filter by the usual methods.

Fresenius' Method.—Extract 20 to 40 grms. of the powdered sample with ether in a continuous extraction apparatus. Dry the residue to remove the ether, shake for fifteen minutes with 120 c.c. of 60 per cent acetone, then allow to stand from twelve to twenty-four hours. Filter, evaporate until free from the acetone, and divide into two portions, a larger and a smaller. Add sufficient acetic acid to dissolve any deposit to the large portion and boil with wool. Boil the wool with dilute acetic acid to remove all natural colouring matter. If the wool is dyed after this treatment, foreign colour is present which can be identified by the usual tests.

To the smaller portion of the aqueous solution, obtained as just described, add an equal quantity of alcohol, heat to dissolve the deposit, divide into four portions, and apply special tests to three of these, keeping the fourth for comparison.

Hydrochloric acid will decolorize the liquid if no artificial colour be present; ammonia will intensify it; and stannous chloride will not affect it. Saffron reacts in a similar way but is only slightly bleached by the acid and is not affected by the other two reagents.

Piutti and Benivoglio Method.—This method is especially in-

tended to detect the four colours forbidden by Italian law and to distinguish them from naphthol yellow S. Boil 50 grms. of the paste in 500 c.c. of water alkalized with 2 c.c. of concentrated ammonia water, add 60 c.c. to 70 c.c. of alcohol and continue to boil for forty minutes. Filter, acidify the liquid with 2 c.c. to 3 c.c. of dilute hydrochloric acid and boil with 5 or 6 strands of fat-free wool, each strand weighing about 0.5 gm. Wash the wool, dissolve the colour in dilute ammonia and dye again. Dissolve a second time in ammonia, then evaporate the solution of the dye to dryness, taking care to avoid the formation of a skin, and take up the residue in water.

If, however, a skin has formed filter and test the insoluble matter for metanil yellow, with dilute hydrochloric acid, and with ammonium sulphide for picric acid.

Add stannous chloride solution and a little sodium hydrate or preferably sodium ethylate to the filtrate. If there is no red coloration, nitro-colours are absent; if in another portion dilute hydrochloric produces no violet colour, metanil yellow is absent and no other test is necessary. When these colours are present acidify the remaining solution with acetic acid, and shake well with carbon tetrachloride, and identify the colour from the following scheme:—

A. Carbon tetrachloride dissolves colour to a colourless solution. Extract with exceedingly dilute ammonia, concentrate and divide into two parts.

- (1) Acidify with hydrochloric acid, then add the 2 drops of stannous chloride and ammonia in excess. A rose-coloured solution and precipitate form.

Naphthol yellow.

- (2) Acidify slightly with hydrochloric acid, add a little zinc dust, then stir. Solution becomes rose-violet.

Victoria yellow.

B. Colour is insoluble in carbon tetrachloride. Evaporate to dryness on a water bath, take up in water and divide into three parts.

- (1) Hydrochloric acid gives a violet coloration.

Metanil yellow.

- (2) Ammonium sulphide gives a red-brown coloration.

Picric acid.

- (3) Stir on a water bath with zinc dust and ammonia, filter, treat with zinc dust and hydrochloric acid, and again filter.

- (a) Potassium hydroxide produces a yellow coloration.

- (b) Ferric chloride gives a yellow coloration.

Naphthol yellow S.

Schmitz-Dumont Test for Tropaeolins.—Add a few drops of dilute hydrochloric acid to a portion of the sample. If there is a resulting reddish or bluish colour, an azo colour or some other coal-tar colour is present.

Test for Turmeric.—Extract the colour from the ground sample by alcohol and identify by the boric acid test.

Other Cereal Flours.—A microscopic examination, preferably side by side with standard preparations, affords the only practicable means of detecting adulteration of most of the cereal flours. The following figures, however, are given as showing the average composition of the chief of these flours, as occasionally information of a useful or confirmatory character may be obtained by a detailed analysis. These figures are those of A. H. Church:—

	Water.	Albumenoids.	Starch.	Fat.	Cellulose.	Mineral Matter.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Wheat	13.0	10.5	74.3	0.8	0.7	0.7
Wheat bran	14.0	15.0	44.0	4.0	17.0	6.0
Oatmeal	5.0	16.1	63.0	10.1	3.7	2.1
Barley	14.6	6.2	76.0	1.3	0.8	1.1
Rice	14.6	7.5	78.0	0.5	0.9	0.5
Rye	13.0	10.5	71.0	1.6	2.3	1.6
Maize	14.5	9.0	64.5	5.0	5.0	2.0
Buckwheat	13.4	15.2	63.6	3.4	2.1	2.3
Peas	14.3	22.4	51.3	2.5	6.5	3.0
Beans	14.0	23.0	52.3	2.3	5.5	2.9
Lentils	14.5	24.0	49.0	2.6	6.9	3.0
Earth nuts	7.5	24.5	11.7	50.0	4.5	1.8

Baking Powders.—It will be convenient to here briefly discuss the composition and analysis of baking powders—not on account of any chemical relationships with the starches, but because they are generally used in conjunction with flour.

Baking powder is an article of considerable interest to analysts working under the Food and Drugs Act, as it was largely due to the decision in *James v. Jones* (58 J. P. 230), following on an earlier case, *Warren v. Phillips* (44 J.P. 61), in which it was held that baking powder was not a food within the meaning of the 1875 Act, that the more extended definition of foods was embodied in the 1899 Act.

Baking powders are mixtures which, when added to flour, etc., give off CO₂ under the influence of moisture, and so enable bread and cakes to be baked without the aid of yeast.

They may be roughly divided into three groups:—

(a) Tartaric acid powder, in which the acid constituent of the powder is either tartaric acid or cream of tartar or a mixture of both.

(b) Phosphoric acid powders, in which the acid constituent is an acid phosphate.

(c) Sulphuric acid powders, in which alum or acid potassium sulphate is the acid constituent.

Bicarbonate of sodium is invariably the alkaline constituent, and nearly all baking powders, contain a good deal of starchy matter as a diluent, and in order to absorb traces of moisture.

The properties of baking powder, from the point of view of the Food

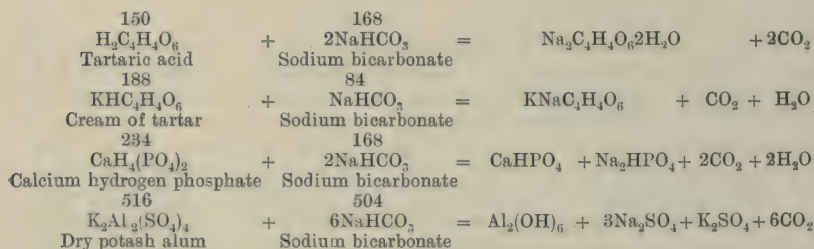
and Drugs Acts, are not merely a question of analysis. If proceedings have to be taken in regard to this substance, it is advisable that they should be under s. 3 of the 1875 Act, when it will be necessary to prove that such constituents as alum, etc., are harmful ingredients.

All the better-class baking powders are tartaric acid compounds. Tartaric acid is more easily soluble than cream of tartar, and powders made of the former evolve their CO_2 more rapidly than those made with the latter; hence in many of the best powders, the two are mixed so as to give an intermediate result.

The following are typical samples of well-known brands of baking powder:—

	1.	2.	3.
	Per cent	Per cent	Per cent
Tartaric acid	20	18	22
Cream of tartar	6	9.5	7
Bicarbonate of soda	25	23.5	27
Starchy matter	49	49	44

The theoretical quantities of the various acid materials necessary for neutralization of the sodium bicarbonate are shown by the following reactions:—



In the analysis of baking powder the following determinations should be made:—

Carbonic Acid.—This is the measure of the strength of the powder, as its value depends on the quantity of gas liberated. Usually the *total* and *available* carbon dioxide are both measured, as, through deficiency in acid ingredients, the whole of the carbonates are not always decomposed when the powder is employed for baking purposes. The total carbon dioxide is obtained by treatment with excess of acid; the available by the use of water.

Any of the usual forms of apparatus for the measurement of carbon dioxide may be used for this purpose. Thus, the well-known Schrödter flask may be used, in which the liberating acid and drying tubes, etc., are all self-contained together with the powder. The loss of weight after reaction is the amount of carbon dioxide evolved. In using an apparatus of this form from 1 to 2 grms. of the powder are

weighed out and transferred to the flask previously charged with dilute sulphuric acid, and concentrated acid for drying the escaping gas; the whole apparatus is weighed and the acid allowed to enter very slowly. Towards the end of the reaction, the flask should be carefully warmed. Finally draw air through in the usual manner, and weigh the flask again. Water must *not* be added to the powder before the reaction is started. To estimate *available* carbon dioxide proceed in the same manner, except that distilled water must be used for liberating purposes, instead of dilute acid.

(2) *Tartaric Acid*.—Weigh out 5 grms. of the powder, transfer to a 500 c.c. flask, and add 100 c.c. of water and 15 c.c. strong hydrochloric acid. When all action has ceased, make up with water to 500 c.c., and allow starch to subside. Filter and take 50 c.c. of the filtrate and add 10 c.c. of 30 per cent potassium carbonate solution. Boil for half an hour and filter into a porcelain dish, concentrate to 10 c.c., add gradually and with stirring 4 c.c. of glacial acetic acid, and then 100 c.c. of 95 per cent alcohol, stirring the liquid until the precipitate floating in it assumes a crystalline appearance. After standing some hours, filter and wash with alcohol until free from acetic acid. Transfer precipitate to a beaker, add water and boil. Titrate the resulting solution with decinormal alkali—1 c.c. of alkali corresponds to 0.0188 gm. of potassium bitartrate (cream of tartar), or 0.0150 gm. of tartaric acid.

(3) *Sulphuric Acid*.—This may be estimated without previous ignition of the powder. Weigh out 0.5 gm. and digest in a beaker with strong hydrochloric acid until the whole of the powder including the starch is dissolved; dilute with water, boil, and add barium chloride in slight excess, allow to stand twelve hours, filter and weigh the BaSO_4 .

(4) *Alumina*.—In the absence of phosphoric acid, from 0.5 gm. to 1.0 gm. may be ignited, extracted with HCl , evaporated to dryness to separate silica, treated with strong hydrochloric acid, again evaporated, filtered, and diluted with water, and alumina precipitated with ammonia, washed, dried, ignited, and weighed. In the presence of phosphoric acid, the following method may be used: Weigh out 5 grms. of the powder, heat until thoroughly carbonized, digest with strong nitric acid, dilute, and filter into a 500 c.c. flask. Wash the residue slightly, transfer it to a platinum dish, dry, burn, add mixed potassium and sodium carbonates, and fuse. Dissolve in nitric acid, evaporate to complete dryness, again dissolve in nitric acid, dilute, and filter into a 500 c.c. flask. The flask will now contain both series of filtrates; make up to 500 c.c. with water. Take 100 c.c. and precipitate with ammonium molybdate and nitric acid, digest and filter. In the filtrate determine alumina by precipitation with ammonia.

(5) *Starch*.—Starch may be determined by treatment with dilute acid so as to convert into glucose, and then estimating this by Fehling's solution.

Phosphoric Acid.—This may be determined by igniting 0.5 gm. of the sample, dissolving in nitric acid, diluting and filtering. The phos-

phoric acid is precipitated by ammonium molybdate solution, the precipitate collected, washed with ammonium nitrate solution, then dissolved in ammonia, precipitated with magnesia mixture, the precipitate washed with dilute ammonia, dried, ignited and weighed as pyrophosphate in the usual manner.

CHAPTER IV.

SPICES, FLAVOURING ESSENCES, ETC.

UNDER the above heading, the ordinary spices and condiments, and allied bodies such as cochineal and turmeric, etc. (used largely for colouring foods), flavouring essences, and vinegar will be considered.

There are a number of useful determinations common to a number of spices, which will be described before the individual substances are dealt with.

Since most of the spices owe their characteristics to essential oils and resins, which are soluble in ether, a determination of the ether extract becomes of importance. But care must be taken to make it clear as to what is meant by "ether extract," since the essential oils are volatile at comparatively low temperatures. In the present chapter the *total ether extract* is the residue left by the spontaneous evaporation of the ether after a Soxhlet extraction, and then left in a sulphuric acid desiccator for twelve hours. The extract is then dried at 100° for several hours, the temperature being slowly raised to avoid oxidation of the oil. The temperature is then increased to 110° till the weight is constant. This gives the *fixed ether extract*, the difference between the two being the *volatile ether extract*.

Starch Determination.—In substances containing much starch, it is generally safe to convert by means of acid and estimate by titration against Fehling's solution (p. 123). But when very small amounts only are present, such as cayenne pepper, the diastatic conversion is safer. Leach recommends that 4 grms. of the powdered sample be extracted with 5 successive portions of 10 c.c. of ether and then with 150 c.c. of 10 per cent alcohol, on a filter paper. The insoluble matter is washed into a 500 c.c. flask (if hydrochloric acid be used to convert the starch) and 200 c.c. of water and 20 c.c. of HCl added. The process is then carried out as described on p. 122.

If the starch is to be determined by the diastase method, the residue is washed into a beaker with 100 c.c. of water and the process carried out as described on p. 176.

Fibre.—This determination is best carried out on the residue left from the ether extract. This residue is boiled for thirty minutes with about 200 c.c. of sulphuric acid (containing 1.5 per cent H_2SO_4). The flask should be well shaken during the boiling, and after thirty minutes the contents are poured on to a filter and the insoluble matter washed with boiling water. The residue is washed back into the flask and boiled with a like quantity of 1.5 per cent solution of NaOH. After

thirty minutes boiling the liquid is filtered, the residue washed with boiling water until the washings are neutral, and then dried. The weight of this residue, less the amount of ash it yields on incineration, is the crude fibre.

Determination of Volatile Oil.—This is described at some length under cloves (p. 224).

The Tannin Value.—Richardson ("U. S. Dept. of Agriculture, Div. of Chem. Bull." 13, 167) determines the tannin value, either in terms of oxygen absorbed, or of quercitannic acid, as follows:—

A standard indigo solution is prepared by dissolving 6 grms. of pure potassium sulphindigotate in 500 c.c. of hot water, cooling, adding 50 c.c. of concentrated sulphuric acid and making up to 1000 c.c.

A standard solution of potassium permanganate is made by dissolving 1.333 grms. of pure potassium permanganate in water to make 1000 c.c.

Two grms. of the substance are extracted for twenty hours with ether. The residue is boiled for two hours with 300 c.c. of water, cooled, the liquid made up to 500 c.c. and filtered. Twenty-five c.c. of this filtrate are run into a 1200 c.c. flask, 750 c.c. of distilled water added and 20 c.c. of the standard indigo solution. Standard permanganate is then run in from a burette, until the colour changes to golden yellow, which indicates the end of the reaction. The number of c.c. of permanganate used is noted (*a*). The titration is repeated on 20 c.c. of indigo solution only. The number of c.c. used is noted (*b*). *a-b* represents the number of c.c. of permanganate used to oxidize the tannin present.

The permanganate solution is standardized against decinormal oxalic acid, so that the amount of permanganate used is easily converted into terms of decinormal oxalic acid. Each c.c. of the latter is equivalent to 0.008 gm. of oxygen absorbed, or to 0.062355 gm. of quercitannic acid.

Winton's Lead Number.—Winton has shown that the amount of precipitate obtained from a solution of certain substances by means of subacetate of lead is fairly constant. The lead number represents the amount of metallic lead precipitated by 100 grms. of the substance (essence of vanilla, for example). Twenty-five grms. of the liquid (or an extract prepared by alcohol of expressed strength, from 25 grms. of a solid), are mixed with 25 c.c. of a standard solution of lead subacetate (made by diluting the ordinary liquor *Plumbi subacetatis* of pharmacy with four times its volume of water) and the whole made up to 100 c.c. and allowed to stand, after well shaking for three hours. It is then filtered, and to 10 c.c. of the filtrate 40 c.c. of water are added, excess of H_2SO_4 and 100 c.c. of methylated spirit. After standing for twelve hours the lead sulphate is filtered off, dried, and weighed. The precipitate $\times 0.6829$ gives the amount of metallic lead in the sulphate (*a*).

Now determine the amount of lead in 2.5 c.c. of the standard lead solution in the same manner (*b*). Then *b-a* represents the lead in the lead precipitate from 2.5 grms. of the substance examined. So that (*b-a*) 40 represents the lead number of the substance in question.

If a solution of normal acetate be used instead of the basic acetate, different results are obtained, so that it is necessary to specify which solution has been used.

GINGER.

Ginger is the rhizome of *Zingiber officinale*, and although employed principally as a spice, is a drug official in the British Pharmacopœia. It is usually sold in the scraped condition, and is so directed to be used as a drug in the Pharmacopœia.

It is not usually necessary to analyse whole ginger, except sometimes in order to determine whether there be any pieces of exhausted ginger present. It is necessary, however, to examine the powdered ginger, which is sometimes adulterated, especially with powdered spent ginger: and preparations sold as prepared from ginger will often be found which have been prepared from capsicum. This is true of many samples of cheap ginger beer, which are frequently rendered hot with capsicum and contain little or no ginger.

Ginger contains an oleo-resin, the resin being of a phenolic nature and representing the pungency of the ginger, whilst the essential oil is responsible for the aroma of the spice.

The presence of exhausted ginger is shown by a low cold water extract, a low soluble ash, and a reduced resin value. Other adulterants of an organic nature are detected by the microscope.

Allen ("Analyst," xix. 124) gives the following tables of analyses of ginger:—

TABLE I.

	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Total ash	3.54	5.23	4.41	5.15	5.53	7.69	5.39	3.61	3.19	2.72	3.52	3.29	4.50
Ash soluble in hot water	2.36	2.59	2.22	2.57	2.87	2.36	—	1.24	1.45	0.69	1.11	0.97	1.37
Alkalinity of soluble ash as K_2O	0.96	0.96	0.29	0.13	0.15	0.20	—	0.27	0.20	0.23	0.23	—	—
Extracted by rectified spirit	7.33	7.70	7.37	6.22	8.45	—	4.65	7.09	—	6.88	7.86	—	—
Extracted by proof spirit	21.64	20.80	10.70	10.45	7.55	21.60	5.85	13.00	16.08	11.78	12.38	—	—
Containing ash	—	2.73	—	—	—	—	—	2.30	2.47	1.91	2.24	—	—
Extracted by cold water	14.57	13.16	14.95	14.55	14.50	14.60	8.14	8.33	9.78	8.51	7.18	8.08	7.39
Extracted by subsequent treatment with proof spirit	9.77	9.59	—	—	—	7.49	—	8.35	8.51	7.76	8.42	—	—
Extracted by subsequent treatment with rectified spirit	1.11	1.28	—	—	—	1.31	—	1.58	0.98	1.28	1.71	—	—
Total extract by the three solvents used consecutively	25.45	24.03	—	—	—	23.4	—	18.26	19.27	17.55	17.31	—	—

TABLE II.

Origin of Ginger.	N.	O.	P.	Q.	R.	S.	T.	U.	V.	W.	Average.	
	Jamaica.	Jamaica.	Jamaica.	Jamaica.	Jamaica.	Cochin.	Cochin.	Cochin.	African.	African.	Per cent	Per cent
Moisture	11.26	10.98	13.95	12.76	13.96	10.64	13.50	13.23	15.97	13.70	13.00	13.00
Total ash	—	3.90	3.29	3.29	3.45	—	3.81	3.62	3.66	3.90	3.66	3.66
Soluble ash	1.70	1.41	3.05	1.75	1.71	1.71	2.03	2.04	2.28	2.41	2.01	2.01
Cold water extract	15.65	13.25	14.40	12.25	11.85	13.00	8.65	11.65	10.80	10.10	12.12	12.12

Richardson has published the following analyses of five samples of ginger :—

Sample.	H ₂ O.	Ash.	Volatile Oil.	Fixed Oil and Resin.	Starch.	Fibre.	Albumenoids.	Nitrogen.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Calcutta	9.60	7.02	2.27	4.58	49.34	7.45	6.30	1.01
Cochin	9.41	3.39	1.84	4.07	53.33	2.05	7.00	1.12
Unbleached Jamaica	10.49	3.44	2.03	2.29	50.58	4.74	10.85	1.74
Bleached	11.00	4.54	1.89	3.04	49.34	1.70	9.28	1.48
"	10.11	5.58	2.54	2.69	50.67	7.65	9.10	1.46

Moisture.—Normal samples of ginger contain from 9 to 12 per cent of moisture. Any great excess of this quantity should not be present, as the spice will have a tendency to become mouldy.

Mineral Matter.—The ash of genuine ginger is about 4 to 5 per cent, sometimes falling as low as 3 per cent or rising to 6.5 per cent. Any slight excess of this amount may be due to the presence of a little dirt, but as ginger of an inferior quality is sometimes limed, any large quantity of lime should be condemned. The following figures represent about twenty-five samples examined by the author :—

I. NORMAL PURE GINGER.

	Jamaica.	Africa.	Cochin.
	Per cent	Per cent	Per cent
Total ash	3.4 to 5.4	4 to 5.8	3.8 to 5.6
Soluble in H ₂ O	1.8 „ 3	1.9 „ 2.9	1.7 „ 3.1
Insoluble in acid	0.3 „ 0.8	0.4 „ 1	0.5 „ 1

II. LIMED GINGERS.

	Per cent
Total ash	5.8 to 9.2 (CaO up to 3.1)
Soluble in H ₂ O	2.4 „ 3.9
Insoluble in acid	0.6 „ 1.1

The amount of ash soluble in water should always be well over 50 per cent of the total. A lower amount indicates the presence of exhausted ginger.

Cold Water Extract.—The sample should be well shaken at intervals with twenty times its weight of water and an aliquot part of the filtered liquid dried at a temperature of 100°, until the loss between the subsequent weighings at intervals of five minutes does not exceed 5 milligrammes. The cold water extract should not be less than 10 per cent, and is usually from 14 to 16 per cent. Exhausted ginger materially reduces this figure.

Carbohydrates.—The carbohydrates, determined by direct inversion of the powder, and estimation by means of Fehling's solution, should be not materially less than 50 per cent nor more than 55 per cent calculated as starch.

Ether Extract.—The oleo-resin, extracted by ether, and dried at about 65° C., until the weight is practically constant, varies from 3 per cent to 6 per cent, rarely rising to 8 per cent (in East Indian ginger).

Good quality Jamaica ginger rarely contains less than 5 per cent of oleo-resin thus extracted. The extraction may be made with alcohol, when slightly higher results will be obtained, but it is less easy to drive off all the solvent than when ether is used. According to Garnett and Grier ("Pharm. Journal," 1909, ii. 159), the pure resin (gingerol) is best determined by exhaustion with ether, recovering the solvent, boiling the residue with several successive portions of petroleum ether and then extracting the petroleum ether (which contains gingerol, volatile and fatty oils and colouring matter) with three successive portions of 60 per cent alcohol. The alcoholic liquid now contains all the gingerol and some impurities. It is washed once with petroleum ether to get rid of traces of fat, the alcohol recovered, and the watery liquid is extracted with three successive portions of ether, which is driven off and the residue weighed. If pure it should be quite soluble in 1 per cent solution of KOH. The amount of gingerol present in genuine ginger usually varies from 1·2 to 2 per cent.

The following may be taken as figures covering most pure samples of ginger :—

	Per cent
Moisture	8·5 to 14·0
Volatile ether extract	1·5 " 3
Non-volatile "	3·0 " 6
Alcohol extract	3·6 " 6·8
Cold water extract	10·5 " 18
Starch	50·0 " 55
Fibre	3 " 7·5
Albumenoids (N × 6·25)	6·5 " 11
Mineral matter	3 " 6
Ash insoluble in HCl	0·02 " 2·3

The following values indicate the composition of ginger after it has been partially exhausted, for essence or ginger ale manufacture :—

	Essence Residue (i.e. Exhausted by Spirit).	Ginger Ale Residue (i.e. Water-Exhausted).
	Per cent	Per cent
H ₂ O	8·02	10·61
Volatile ether extract	0·13	1·61
Non-volatile "	0·54	3·86
Alcohol "	1·52	4·88
Cold water "	16·42	6·15
Starch	—	54·57
Fibre	—	5·17
Albumenoids	—	6·94
Ash	5·05	1·1 to 2·12
" soluble in H ₂ O	3·55	0·2 " 0·59
" insoluble in HCl	1·50	0·18

Dyer and Gilbard ("Analyst," xviii. 197) first called attention to the water-soluble ash as a reliable means of indicating water-exhausted ginger. Six samples of pure and exhausted ginger gave the following results:—

		Total Ash	Water Sol. Ash.	Alcohol Ext. After Ether Ext.
		Per cent	Per cent	Per cent
Pure ginger	Highest	4.1	3.0	3.8
	Lowest	3.1	1.9	2.1
	Average	3.8	2.7	2.8
Exhausted ginger	Highest	2.3	0.5	1.5
	Lowest	1.1	0.2	0.8
	Average	1.8	0.35	1.2

Microscopic Examination.—The starch grains are more or less sack-shaped, with somewhat rounded ends. The smaller grains are

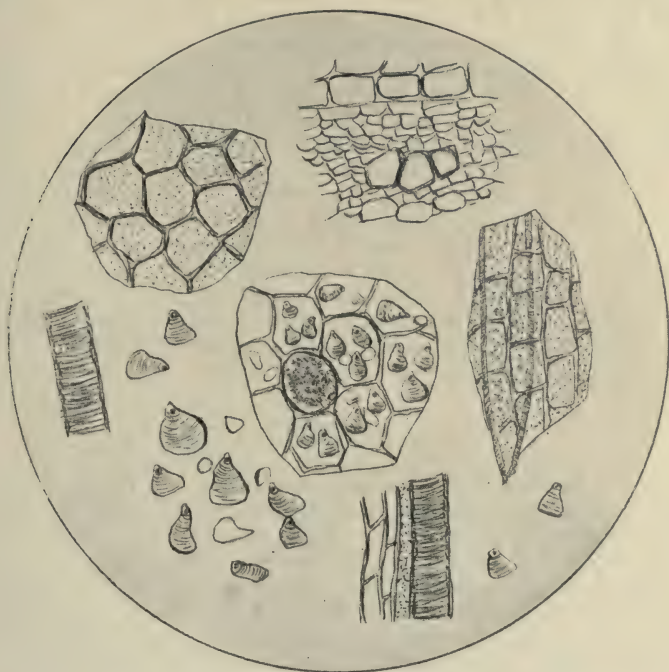


FIG. 22.—Powdered ginger.

nearly circular. The hilum and striations are almost invisible, the former being close to the pointed extremity of the grains. The grains

measure from 20 to 30 μ , although a few will be found measuring so little as 15 μ or so much as 50 μ .

An examination should be made, after the starch has been removed, by boiling 5 grms. with 50 c.c. of 5 per cent hydrochloric acid. The tissues should be washed with water and then examined in a 50 per cent solution of chloral hydrate. Characteristic fibres will be found, with spirally arranged pits.

The Detection of Capsicum in Ginger Preparations.—Garnett, Grier and La Wall ("Analyst," xxxiv. 321) recommend the following process. The ginger ale, etc., is warmed to expel CO_2 , and if alcohol be present this is driven off also. The aqueous residue is acidified with dilute sulphuric acid and shaken with 50 c.c. of ether for a minute. If the residue from the ether, which is allowed to evaporate spontaneously, weighs less than 10 milligrammes it is treated with 2 c.c. of $\frac{\text{N}}{2}$ alcoholic caustic potash solution. An additional 1 c.c. of alkali is added for each further 10 milligrammes. The mixture is transferred to a test tube fitted to a reflux tube and gently boiled for thirty minutes in a water bath. The alcohol is drained off, and the test tube is half filled with water, and the liquid well shaken with half its volume of ether. The ether is separated and evaporated. If the residue has a hot, pungent taste, capsicum is present—the phenolic constituents of the ginger being retained by the potash. One part of capsicum in 10,000 of water can thus be detected. Samples of gingerine are similarly examined, using 50 milligrammes of the sample.

Nelson ("J. Ind. and Eng. Chem." 1910, 2, 419) prefers to take the ether extract from 100 c.c. of a beverage, which is first heated to drive off alcohol, and evaporate it with 10 c.c. of twice normal alcoholic potash. About 7 mgs. of manganese dioxide and 5 c.c. of water are added, and the whole heated till volatile oils are driven off. The cold liquid is acidified with dilute H_2SO_4 , and at once extracted with petroleum ether. The solvent is evaporated and the residue touched with the tip of the tongue, when the burning taste of capsicum, if present, cannot be mistaken.

PEPPER.

Pepper consists of the not quite ripe fruit of *Piper nigrum*, cultivated in India and the islands of the Malay Archipelago. Black pepper consists of the entire fruit, whilst white pepper consists of the berries deprived of the outer portion of the pericarp.

Constituents.—Pepper consists of the usual plant tissues, together with a small amount of an essential oil (from 1 to 2 per cent), a small amount of a bitter, hot, pungent resin, and from 3 to 7 per cent of the alkaloid piperine, with possibly a small quantity of piperidine. It contains an appreciable amount of pepper starch.

The quality of pepper depends almost entirely on the amount of resin and alkaloid, although the flavour is influenced by the amount of essential oil.

A number of samples of various origins have been examined by the author, and the following results obtained :—

	Moisture.	Ash.	Watery Extract.	Alkaloid.	Resin.
	Per cent	Per cent	Per cent	Per cent	Per cent
Penang pepper (10 samples)	8.35 to 9.96	4 to 6	17 to 19.5	5 to 6.2	2 to 2.2
Sumatra pepper (8 samples)	8.5 „ 11.0	4.5 „ 6.1	17 „ 20.3	4.3 „ 5	2 „ 2.3
Malabar pepper (10 samples)	9 „ 10.8	4.8 „ 5.7	18 „ 20	4.0 „ 5.8	1.9 „ 2
White pepper (origin unknown)	10 „ 12	1.2 „ 1.6	20 „ 22	4.3 „ 5.3	2 „ 2.3

The following table is abridged from one by Heisch ("Analyst," xi. 188).

	Ash in Moisture Free Pepper.			Per cent on the Moisture and Ash Free Pepper.		
	Total.	Sol. in H ₂ O.	Insol. in HCl.	Starch.	Alcohol Extract.	Piperine.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Black peppers	4.35 to 8.99	1.54 to 3.34	0.04 to 4.38	48 to 57	11.6 to 16.2	4.05 to 9.38
White peppers	1.28 „ 3.78	0.16 „ 0.61	0 „ 0.69	76 „ 85	9.2 „ 10.6	4.50 „ 6.14
Long peppers	12 „ 13.5	2.3 „ 2.4	3.7 „ 5.7	46 „ 59	8.3 „ 8.5	1.70 „ 1.71
Black pepper husks	11.9	2.12	3.41	41.7	13.8	4.84
¹ Siftings before grinding	51.4	1.02	43.90	30.66	7.5	1.15

Richardson gives the following values :—

	H ₂ O.	Ash.	Volatile Oil.	Piperine and Resin.	Alcohol Extract.	Starch.	Fibre.	N.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Black	8.91	4.04	0.70	7.29	—	36.52	10.23	1.57
„	8.29	4.70	1.69	7.72	6.06	37.50	10.02	2.02
„	9.83	3.70	1.60	7.15	5.74	37.30	10.02	1.93
White	9.85	1.41	0.57	7.24	—	40.61	7.73	1.83
„	10.60	1.34	1.26	7.76	2.57	43.10	4.20	1.90

Gladhill ("Amer. Jour. Pharm." 76, 71) gives the following analyses of genuine samples of pepper :—

¹ This cannot be considered as pepper at all.

Black Pepper.	Ash.				Ether Extract.				Piperine.				Oleo-resin.						
	1.	2.	3.	4.	1.	2.	3.	4.	1.	2.	3.	4.	1.	2.	3.	4.			
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent			
Singapore	3.5	3.7	4.2	4.5	9.76	8.76	9.52	9.60	7.13	7.68	6.58	7.33	2.63	1.08	2.94	2.27			
Tellicherry	4.7	4.8	3.8	4.5	8.34	8.85	7.26	7.62	5.91	6.02	6.56	6.82	2.43	2.83	0.70	0.80			
Aleppo	4.7	4.7	—	—	9.65	9.47	—	—	7.70	6.75	—	—	1.95	2.72	—	—			
Trang	3.9	3.8	—	—	8.44	8.83	—	—	5.12	5.61	—	—	3.32	3.22	—	—			
Lienburg	3.8	4.0	3.6	4.0	8.70	9.48	8.83	8.78	6.50	6.28	5.98	6.31	2.20	3.20	2.85	2.47			
Lampong	5.0	5.5	5.4	5.2	8.92	10.31	8.76	9.58	7.76	8.30	7.00	7.28	1.16	2.01	1.76	2.30			
W. C. Sumatra	4.3	4.0	—	—	9.28	9.22	—	—	7.00	6.68	—	—	2.28	2.54	—	—			
Acheen A	4.3	4.5	4.0	4.7	10.06	10.10	9.80	9.20	7.56	7.96	7.67	7.10	2.50	2.14	2.13	2.10			
" C	5.5	5.2	—	—	10.46	10.46	—	—	10.02	9.94	—	—	0.44	0.52	—	—			
White Pepper.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
Coriander	1.0	0.8	1.0	0.8	8.27	11.68	8.16	7.90	6.81	9.00	7.16	6.84	1.46	2.68	1.00	1.06			
Singapore	1.1	1.0	1.2	—	8.78	8.45	8.20	—	7.26	6.78	7.20	—	1.52	1.67	1.00	—			
Penang	2.1	2.8	2.6	—	7.04	7.20	6.80	—	5.74	6.76	5.83	—	1.30	0.44	0.97	—			
Decorticated	1.9	0.8	1.2	—	7.64	6.60	7.26	—	6.25	6.30	7.02	—	1.39	0.30	0.24	—			
Ether Extracts.																			
Ash.				Ether Extracts.				Ether Extracts.				Ether Extracts.				Ether Extracts.			
1.	2.	3.	4.	5.	6.	7.	8.	1.	2.	3.	4.	5.	6.	7.	8.	1.	2.	3.	4.
Hull ¹	9.4	9.6	8.9	7.0	7.7	8.3	8.8	6.39	6.39	8.88	8.93	5.36	5.46	5.00	5.92	—	—	—	—

¹ Pepper hull contained no piperine.

The following are numerous analyses of various American chemists (Brooks, "Federal Spice Standards, 1909"), of black, white, and long peppers.

BLACK PEPPERS.

Variety, Etc.	Moisture.	Volatile Ether Extract.	Non-volatile Ether Extract.	Starch (Diastase Method).	Crude Fibre.	Parts Nitrogen in 100 Parts Non-volatile Ether Extract.	Total Ash (Mineral Matter).	Ash Insoluble in Hydrochloric Acid.	Weight in Grams. Per 100 Berries.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Singapore (14 samples)									
Minimum . . .	8.20	0.99	6.57	33.75	10.02	3.91	3.09	0.07	3.90
Maximum . . .	12.43	1.94	7.92	39.66	13.82	4.22	4.95	0.56	5.46
Tellicherry (7 samples)									
Minimum . . .	8.42	0.65	6.72	36.03	11.98	3.88	4.06	0.00	4.13
Maximum . . .	11.86	1.55	7.02	41.75	13.21	4.14	4.69	0.10	5.11
Aleppo (5 samples)									
Minimum . . .	8.46	1.12	7.48	34.65	11.36	3.72	4.74	0.07	4.29
Maximum . . .	10.01	1.90	8.87	41.60	13.01	3.98	5.02	0.30	5.41
Malabar (2 samples)									
Minimum . . .	9.47	1.04	6.10	36.84	9.68	3.86	3.45	0.09	4.23
Maximum . . .	10.53	1.51	7.71	44.83	12.78	4.00	4.40	0.20	5.74
Lamong (10 samples)									
Minimum . . .	8.09	1.11	6.81	33.41	10.25	3.82	4.86	0.48	3.32
Maximum . . .	12.17	2.10	9.05	39.46	13.50	4.27	6.52	1.80	3.79
Trang (5 samples)									
Minimum . . .	8.09	1.22	6.60	35.73	10.58	3.79	3.43	0.33	3.82
Maximum . . .	11.57	1.60	6.97	41.00	13.11	4.10	4.16	0.41	4.13
Acheen A (3 samples)									
Minimum . . .	8.73	1.09	9.17	28.00	13.07	4.02	5.04	0.48	3.20
Maximum . . .	12.09	1.71	10.44	33.72	16.97	4.21	6.49	0.96	3.69
Acheen B (3 samples)									
Minimum . . .	8.89	1.15	9.03	25.09	14.09	4.06	5.80	1.15	2.52
Maximum . . .	12.95	2.07	9.16	33.08	18.84	4.13	6.62	1.36	2.79
Acheen C (4 samples)									
Minimum . . .	9.62	1.28	7.99	22.05	16.40	3.94	6.10	1.00	2.12
Maximum . . .	12.33	2.05	9.64	33.38	18.25	4.18	8.04	2.59	2.82
Acheen D (2 samples)									
Minimum . . .	10.03	1.66	8.24	28.00	17.98	4.05	6.75	1.52	—
Maximum . . .	10.06	1.98	8.81	28.40	18.89	4.15	7.00	1.62	2.46
Mangalore (3 samples)									
Minimum . . .	8.53	1.50	6.81	34.62	10.00	3.46	4.03	0.05	8.57
Maximum . . .	11.61	1.87	9.08	36.95	10.42	4.06	4.74	0.19	9.72
Shot pepper (3 samples)									
Minimum . . .	8.40	1.16	6.66	33.19	10.58	3.29	3.66	0.20	4.84
Maximum . . .	11.50	1.41	7.49	38.60	13.04	4.07	4.15	0.28	6.00

WHITE PEPPERS.

Variety, Etc.	Moisture.	Volatile Ether Extract.	Non-volatile Ether Extract.	Starch (Diastase Method).	Crude Fibre.	Parts Nitrogen in 100 Parts Non-volatile Ether Extract.	Total Ash (Mineral Matter).	Ash Insoluble in Hydrochloric Acid.	Weight in Grams. Per 100 Berries.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Singapore (9 samples)									
Minimum	8.15	0.90	5.68	53.11	3.39	4.22	0.94	0.06	4.35
Maximum	13.82	1.66	7.94	59.34	6.10	4.35	1.61	0.20	5.20
Penang (10 samples)									
Minimum	8.04	0.62	5.65	48.88	3.70	4.02	2.15	0.05	4.79
Maximum	14.19	1.64	6.50	54.74	7.65	4.37	4.28	0.28	5.62
Siam (5 samples)									
Minimum	8.66	0.58	5.71	55.01	3.49	4.20	1.26	0.04	4.49
Maximum	14.47	1.37	6.81	56.33	3.91	4.48	1.77	0.22	5.48
Tellicherry (1 sample)	10.49	1.15	6.09	57.09	3.39	4.31	0.86	0.07	5.40
Decorticated (8 samples)									
Minimum	8.14	0.49	5.96	57.38	0.10	4.23	1.00	0.00	2.56
Maximum	13.34	1.50	7.26	63.60	2.07	4.53	2.24	0.15	3.47
Coriander (1 sample)	10.22	0.85	6.48	56.60	4.14	4.15	1.03	0.05	4.22

LONG PEPPERS.

	Minimum.	Maximum.
	Per cent	Per cent
Moisture	8.43	10.13
Volatile ether extract	0.79	1.55
Non-volatile ether extract	5.71	7.53
Starch (diastase method)	28.43	45.87
Crude fibre	5.76	10.01
Total ash	5.93	14.39
Ash insoluble in acid	0.22	5.92
Parts nitrogen in 100 parts non-volatile ether extract	3.12	3.56

The following determinations should be made:—

Moisture.—About 5 grms. of pepper coarsely bruised if the whole berries are being examined, or of the powdered pepper, are dried in a water-oven to constant weight. Any excess over 13 per cent may be regarded as moisture purposely added. The average value is from 8 to 11 per cent.

Mineral Matter.—The portion used to determine the moisture is ignited in the usual manner, and the ash weighed. It should never exceed 8 per cent, and this figure may be regarded as rather excessive, 5 to 6 per cent being the average. Long pepper, however, yields an ash of up to 14 per cent. Since the husks contain up to 14 per cent of mineral matter, the white or decorticated pepper always has a lower

ash value than black pepper. Pepper is said in most text books to be adulterated with chalk, barium sulphate, and such mineral additions. In ground pepper these adulterants are very rarely met with, but in whole pepper, the author has on several occasions met with samples which are not decorticated, but consist of black pepper carefully coated with kaolin and sold as white pepper. The kaolin is rendered adherent with a little gum tragacanth or similar substance, and the black pepper can be treated so well in this manner as to deceive the casual purchaser. Naturally a very high ash value is found in such samples.

The Ash of Pepper.—An average black pepper ash contains the following:—

	Per cent
Potassium (as K_2O)	24
Sodium (as Na_2O)	3.5
Magnesia	12.0
Lime	12.0
Iron	0.25
P_2O_5	8
SO_2	9
Chlorine	8
Silica	6

The following values are representative of normal peppers.
(Leach):—

	Total Ash.	Soluble in Water.	Insoluble in HCl.
	Per cent	Per cent	Per cent
Black	3.49	2.10	0.12
"	4.21	2.75	0.01
"	6.05	2.37	1.06
"	5.04	2.78	0.48
White	1.06	0.47	0.01
"	1.33	0.33	0.09
"	1.47	0.38	0.10
"	2.84	0.65	0.15
Long pepper	5.93	4.20	0.22

Resin and Alkaloid.—The pepper is ground to a fine powder, and well exhausted with strong methylated alcohol. The alcohol is evaporated at about 60° , and the extract left in a desiccator for 24 hours. The extractive matter is almost entirely alkaloid and resin. A separation, not very exact, but sufficiently accurate for practical purposes, may be affected by treating the extract with a 5 per cent solution of caustic potash, pouring off the aqueous liquid, washing with water and redissolving the alkaloid in 95 per cent alcohol. The solution is filtered, the alcohol evaporated at 60° , and the residue allowed to stand in a desiccator for twenty-four hours, and weighed. According to Stevenson ("Analyst," XII. 144) black pepper contains up to 7.14 per cent of piperine, and about 1.5 per cent of resin, whilst white pepper contains up to 6.47 per cent of piperine and 0.69 per cent of resin.

Stoddart ("Analyst," XIV. 37) has recorded a sample of pepper

containing a mixture of starch, barium sulphate, chalk and lead chromate. The latter is said to have been added to improve the colour. Such an adulteration is not likely ever to be met with again.

Watery Extract.—Five grms. of the pepper should be powdered and shaken at frequent intervals for twenty-four hours, with 100 c.c. of water, the whole filtered, the residue washed with two or three portions of about 20 c.c. of water, the washings added to the filtrate, and the liquid evaporated on a water bath. The extract should amount to 17 to 22 per cent.

Determination of Starch.—Lenz regards the determination of the starchy matter in pepper as of the greatest importance. He carries out the estimation in the following manner:—

About 3 grms. to 4 grms. of the powdered sample are heated with 250 c.c. of cold water for a few hours with constant agitation. The solution is filtered and the residue washed with cold water, and the insoluble matter washed into a flask and diluted to 200 c.c. with water. Twenty-five c.c. of a 25 per cent solution of hydrochloric acid are added and the flask, attached to a tube or condenser, is heated with occasional agitation, in a water bath for three hours. On cooling, the liquid is made up to 500 c.c. after neutralization with caustic soda solution. The copper-reducing sugars are then determined by means of Fehling's solution.

Genuine pepper gives a result equivalent to at least 50 per cent of starch calculated on the ash and moisture free pepper, whereas most adulterants except starch itself give much lower results. Röttger gives 57 per cent to 60 per cent as the average value for black pepper and 59 per cent to 74 per cent for white pepper.

This method, of course, involves the determination of all bodies which yield sugars or reducing substances on hydrolysis by acids. When the diastase conversion method is used the results will be from 5 per cent to 10 per cent lower, averaging from 40 per cent to 48 per cent on the moisture and ash free pepper.

Determination of Nitrogen.—The most satisfactory method for the determination of the nitrogen in pepper, which varies from 1.95 per cent to 2.55 per cent is the Gunning-Arnold modification of Kjeldahl's process: 1 gm. of the sample in powder is mixed with 1 gm. each of copper sulphate and red oxide of mercury and about 16 grms. of K_2SO_4 ; 25 c.c. of H_2SO_4 are added and the digestion and distillation carried out in a 600 c.c. flask. After about four hours digestion at boiling temperature, the liquid is cooled and 300 c.c. of water and 50 c.c. of a 4 per cent solution of potassium sulphate are added, and finally enough NaOH solution to render the liquid alkaline. The ammonia produced is then distilled in the usual manner for Kjeldahl's determination.

Adulteration of Pepper.—Such adulterations as chalk, sand, clay, barium sulphate, and the like are readily indicated by the high ash value of the sample. Further, as the mineral matter of pepper is in combination with organic matter, it is not nearly so heavy as added mineral matter. By shaking—say 5 grms.—of the powder in a separating funnel with chloroform, the added mineral matter, with a

small quantity of the natural husk material, rapidly sinks to the bottom and may be drawn off and examined. The presence of added mineral matter is not uncommon even in the whole peppercorns. Inferior black pepper is sometimes coated with lime and mixed with white peppercorns and sold as white pepper.

Commercial adulterated pepper frequently contains ground rice or other starchy matter. These starchy adulterants are recognized by a microscopical examination of the sample, together with a low ash value, a high starch content, and a low result for resin and alkaloid.

Exhausted ginger has been found in pepper. This may be recognized by the appearance of the ginger starch under the microscope.

Perhaps one of the most common adulterants found in pepper of recent years is ground olive stones, originally known under the unsuitable name of poivrette.

According to Campbell Brown the composition of ground olive stones is as follows, a comparison also being made with ground almond shells :—

	White Poivrette.	Black Poivrette.	Almond Shells.	Olive Stones.
	Per cent	Per cent	Per cent	Per cent
Ash	1.33	2.47	2.05	1.61
Starch	none	none	none	none
Soluble in dilute HCl . . .	38.3	34.5	23.5	39.1
Insoluble in acid and alkali.	48.5	48	51.7	45.4

No starch is present in this adulterant, but substances capable of inversion are present and an apparent starch content of 10 per cent will be found.

The following figures are of interest as showing the general character of some of the possible adulterants of pepper :—

	Cocoanut Shells.	Almond Shells.	Date Stones.	Walnut Shells.
	Per cent	Per cent	Per cent	Per cent
Ash (total)	0.54	2.90	1.25	1.40
„ H ₂ O soluble	0.50	2.40	0.76	0.8
„ insoluble in acid	0.0	0.05	0.04	0.0
Ether extract	0.25	0.75	8.5	0.65
Alcoholic extract	1.2	5.20	16.7	1.90
Nitrogen	0.15 to 0.2	0.2 to 0.3	0.85	0.3

The shells of black pepper, which have been removed in the preparation of white pepper, are sometimes ground in with the pepper of commerce. It would be difficult to say this is an adulteration in a legal sense, but to the analyst it would be indicated by an excess of the characteristic stone cells seen under the microscope, and by a high ash value.

The estimation of pentosans (see under cocoa, p. 23) is of value.

in indicating the presence of both pepper and other shells in ground pepper. According to Hehner ("Analyst," xxiv. 181) genuine white pepper yields 1.68 per cent of pentosans (5.54 per cent of crude fibre); genuine black pepper, 4.58 per cent of pentosans (9.91 per cent of crude fibre); and pepper husks 10.24 per cent of pentosans (14.63 per cent of crude fibre).

Numerous colour reactions have been suggested to detect the presence of ground olive stones in pepper. The sample may be mixed into a paste with dilute caustic soda solution, the paste diluted with water and the residue washed by repeated decantation. Particles of ground olive stones are coloured bright yellow, whilst the darker particles are black pepper husk. Bleached pepper husk, however, takes on a very similar colour.

Chevreau utilizes the fact that an acid solution of aniline colours the sclerenchymatous tissues yellow, but leaves the other tissues unaffected. The sample is moistened with a solution of aniline in three times its volume of strong acetic acid. To the naked eye no change is apparent, but under the microscope only a few isolated yellow cells can be found, if the pepper is pure, whilst in the presence of olive stones or almond shells, the sample becomes of a yellow colour and numerous yellow cells can be observed. Martelli prefers to digest 1 gm. of phloroglucinol in 50 c.c. of hydrochloric acid for a few days and decant the clear liquid. The sample is covered with a reagent and heated for a few minutes. Olive stones and similar tissues give a reddish-violet colour whilst pepper is only tinged yellow or faintly brown. If water be added and decanted the stained woody tissue is left as a sediment.

For other colour reactions, reference may be made to a note by Pabst ("Journal Soc. Chem. Industry," 9, 770) who recommends a solution of dimethyl-*p*-phenylenediamine, which colours the ground olive stones a carmine red colour, leaving the pepper almost unchanged. Thalline sulphate (a 1 per cent solution in water) stains ground olive stones a fine orange colour, leaving the pepper but little affected.

To form an approximate judgment as to the amount of ground olive stones in a sample of pepper, the amounts of ash and of starch, both of which will be reduced, are taken into account, together with the amount of woody fibre. The last named may be determined in the following manner:—

Two grms. of the powder previously exhausted by ether are boiled with 200 c.c. of 1.5 per cent H_2SO_4 for 30 minutes, and the residue filtered off. This is now boiled for 30 minutes with 1.5 per cent solution of caustic soda, and the fibre filtered off, washed with water, dried and weighed. This should be done under a reflux condenser, and the flask requires very firm attachment owing to the frequent bumping that takes place. The residue is filtered through a dried tared filter, washed with hot water, dried and weighed. The following are the average amounts of woody fibre obtained:—

	Per cent
White pepper	3 to 9.5
Olive stones	60 " 75
Black pepper	10 " 18
Almond shells	65 " 80

It will be convenient to here mention long pepper, the fruit of *Piper officinarum* (Malay) and of *P. longum* (Bengal and Philippine Islands).

This is a spice used in pickles, but rarely found in retail commerce. It is seldom, if ever, found in the powdered form, so that adulteration is most exceptional. J. Campbell Brown gives the following analyses of three samples of long pepper:—

	1.	2.	3.
	Per cent	Per cent	Per cent
Total ash	8.91	8.98	9.61
Ash insoluble in HCl	1.2	1.1	1.5
Starch (and other sugar-producing bodies)	44.04	49.34	44.61
Albuminous matter soluble in KOH	15.47	17.42	15.51
Cellulose	15.70	10.50	10.73
Alcohol extract	7.7	7.6	10.5
Ether extract	5.5	4.9	8.6
Nitrogen	2.1	2.0	2.3

The Microscopic Examination of Pepper.—A small quantity of the

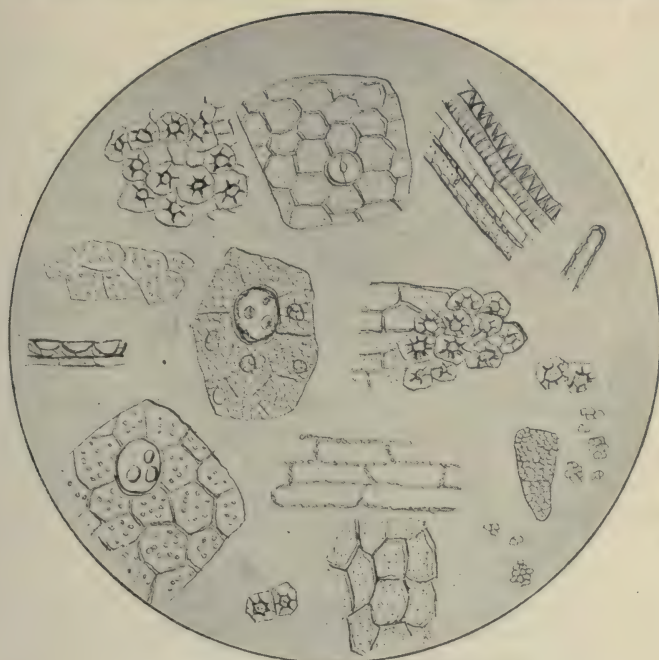


FIG. 23.—Powdered black pepper.

powder should be examined in dilute glycerine (1 in 4 of water). A

large portion of the powder consists of fragments of connected polygonal cells (of the perisperm) packed with minute starch granules: many of the cells are broken, and tiny starch grains, isolated, or in connected groups, can be identified. Irrigation with iodine solution will be useful to identify the starch grains. A second preparation should be made by boiling for ten minutes a little of the powder with a dilute solution of hydrochloric acid (about 0.5 per cent). The powder should then be washed with dilute solution of potash (1 per cent) and then with water. It is best mounted in chloral hydrate solution (70 per cent). The starch is destroyed and the cell structures are observed to better advantage. Thick-walled sclerenchymatous cells, fibrovascular bundles, with spiral vessels and empty parenchymatous cells are all to be found. The general appearance of ground pepper under the microscope is as shown below.

Hanausek ("Zeit. F. Untersuch. der Nahr. und Genussmittel," 1898, 490,) records a case of adulteration with ground coriander seed. The characteristics of this spice under the microscope are (1) bundles of corrugated bent fibrous cells, (2) coarse parenchyma overlaid with narrow cells of a yellow colour, with parallel walls, (3) colourless cellular parenchyma enclosing crystals in rosettes.

CAYENNE PEPPER.

Cayenne pepper consists of the powdered fruits of various species of capsicum, of which *Capsicum pubescens* is the principal. *C. fastigiatum*, *C. annuum* and *C. minimum* are other species used. The so-called "tasteless cayenne" is derived from a species of Pimento grown chiefly in Spain and is without any pungency. It is used principally as a colouring material for certain types of sweet pickles, and for stuffing olives, or for imparting an orange colour to canaries, who eat it mixed with their ordinary food.

The capsicum pods, in their entire condition, are known as chillies.

As a drug, *Capsicum minimum* is official in the British Pharmacopœia. The only standard there laid down for the whole fruit, is that it should not yield more than 6 per cent of ash.

Cayenne pepper is not very frequently adulterated, and certainly never with the absurd adulterations usually enumerated in text books, such as cinnabar.

It owes its virtue to the presence of an oleo-resin of a somewhat complex character, and to a definite crystalline body isolated by Thresh, and named by him capsaicin. Its formula is represented empirically, at all events, by $C_{18}H_{28}NO_3$ and it melts at 64.5° . It is present to the extent of about 0.05 per cent to 0.14 per cent.

On heating the minutest trace of cayenne pepper, these acrid principles volatilize and produce such an intensely irritating vapour which so affects the throat that the presence of even a minute quantity of cayenne could scarcely be overlooked.

According to Richardson, the following represents the average composition of cayenne pepper:—

	Seed.	Pericarp.	Whole Fruit.
	Per cent	Per cent	Per cent
Water . . .	8.12	14.75	11.94
Albumenoids . . .	18.31	10.95	13.88
Ether extract . . .	28.54	5.48	15.26
Fibre . . .	17.50	23.73	21.09
Ash . . .	3.20	6.62	5.20
Nitrogen . . .	2.93	1.71	2.22

Wynter Blyth gives the following figures as the means of several samples :—

	Per cent
Aqueous extract of dried pepper	32.1
Alcoholic extract of „ „	25.79
Benzol extract	20.00
Ether extract	10.43
Ash	5.69 (soluble 3.32)
Nitrogen	2.04

The ether extract is here far too low, and this figure must not be taken as at all representative.

Mineral Matter.—The ash of cayenne pepper should vary between 4 per cent and 7 per cent, with a maximum of 1.2 per cent of siliceous matter insoluble in hydrochloric acid. Rarely, a sample may contain 7.5 per cent of mineral matter. Over 50 per cent of the ash is soluble in water.

Barium salts are sometimes found in adulterated cayenne, which has been artificially coloured with a coal-tar lake on a barium base in order to improve the colour.

Oleo-resin.—The amount of oleo-resinous matter obtained by various solvents gives a good indication of the character of the sample, and its determination will guard against the presence of exhausted cayenne.

It must be remembered, however, that capsicum fruit contains much fat, so that all extracts with organic solvents are merely mixtures of so much oleo-resin, fat, and other extractive matters. The most reliable figures from the extractive matter of genuine capsicum are those of A. W. Gerrard. He has carried out a number of experiments with the following results :—

Ten grms. of capsicum in No. 60 powder were packed in each of six percolators, composed of glass syringe tubes, and slowly percolated respectively with ether, 90 per cent alcohol, benzene, petroleum ether, bisulphide of carbon, and chloroform until 100 c.c. of percolate had collected from each. The percolates were evaporated over a steam bath until the solvent was quite removed. The residues obtained were weighed, and are given here as percentages :—

Solvents Used.	Percentage Yield of Extract.
Ether	18.2
Alcohol, 90 per cent	26.4
Benzene	18.6
Petroleum ether	16.4
Bisulphide of carbon	16.7
Chloroform	17.5

The physical characters of each extract differed somewhat; all of them on standing twenty-four hours deposited a soft granular fat, and separated a fluid dark red resin. The fat yielded from the carbon bisulphide was somewhat crystalline, and in the resinous portion of the extract numerous small crystals were seen floating. The palest coloured extract was obtained by petroleum ether, which solvent does not readily remove the colour from capsicum. To make certain that the powdered capsicum in each case had been properly exhausted, small portions of each marc were tested, and except in the case of the alcohol-treated marc, all were found to possess much pungency. The alcoholic marc, though slightly warm to the taste, might certainly be considered as practically exhausted. It is thus evident that a much larger yield of extract is obtained by alcohol than by the other solvents, the alcohol giving 26.4 per cent against an average of 17.5 per cent from the others, a difference in favour of the alcohol of about 35 per cent. The dried mares or residues of the previous extractions, except that with alcohol, were again packed in the percolators and treated with 90 per cent alcohol until 100 c.c. had been collected; on evaporation of the alcohol there was obtained in each case a brown, resinous, strongly pungent residue, in the following proportions:—

		Per cent
From the ether	marc	7.9
“ “ benzene	“	7.5
“ “ petroleum ether	“	9.0
“ “ bisulphide of carbon	“	7.2
“ “ chloroform	“	7.4

By adding these figures to the previously obtained figures, under their proper solvents, we get approximately the same amount of extract as when alcohol alone is employed. It is thus clear that alcohol is the most powerful and perfect solvent of capsicum.

From numerous analyses by the author, these figures have been fully confirmed, and the following may be taken as the usual limits for the extractive matter of genuine cayenne pepper (Gerrard's figures appear to have been obtained from *Capsicum minimum*):

	Per cent
Alcoholic extract	23.5 to 27.5
Ether extract	15.5 „ 19
Benzene extract	16.0 „ 18.6
CS ₂ extract	15.0 „ 19
Chloroform extract	16.0 „ 18.5

The following may be taken as the average values for the usual analytical figures for cayenne pepper:—

	Per cent
Moisture	3.5 to 7.5
Ash	5.0 " 7.2
„ insoluble in HCl	0.05 " 0.3
„ soluble in H ₂ O	3.3 " 0.3
Non-volatile ether extract	15.5 " 19
Volatile	0.7 " 2.8
Starch	0.8 " 1.5
Fibre	20 " 25
Albumenoids	13 " 15
Aqueous extract	31 " 34
Alcoholic „	25 " 30

Microscopical Examination.—The best method of examining cayenne pepper is to defat some of the powder with ether-alcohol, and then mount in chloral hydrate. This should be done, after examining some of the original powder in water, when the numerous red globules of oleo-resin are observed.

In the defatted preparation, several characteristic elements are easily observed.

It is remarkable that text books—even those published during the last year—consistently draw attention to the absence of starch. This statement appears to be due to an old statement of Dr. Hassel's and has been faithfully reproduced ever since.

It is not, however, a fact. Very small starch grains are to be found in all cayenne pepper. The author has powdered numerous species of pods, and defatted the powder. The specimens on staining with weak iodine show fairly numerous very small starch grains embedded in the cells. A comparison of such a preparation of a commercial sample, with a standard sample, will at once indicate whether any added starchy matter is present.

Wallis ("Pharm. Journ." [4], 15, 3) thus summarizes the microscopic characters of the three species of capsicum met with in commerce:—

	C. Minimum.	C. Annum.	Japanese Chillies.
<i>Epidermis.</i>	Thick and straight-walled rectangular cells with few pits; often arranged in groups of 5 to 7 in a row and with a uniformly striated cuticle. Size of cells, 25 μ to 60 μ in either direction.	Irregular polygonal cells with evenly thickened walls, traversed by numerous well-marked simple pits. The cuticle shows striated ridges. Size of cells, 50 μ to 100 μ long, and 25 μ wide.	Cells with strongly thickened walls and a radiated lumen. The pits only rarely penetrate the whole thickness of the wall. No visible striation. Size of cells, 30 μ to 80 μ long, and 15 μ to 45 μ wide.
<i>Hypodermis.</i>	Delicate thin-walled cellulose cells.	Several layers of cuticularized collenchymatous cells, having a rounded outline and very few pits.	A singular layer of regular polygonal cells with cuticularized fairly thick walls, traversed by numerous pits, which gave them a beaded appearance.

Note.—For the detection of cayenne in ginger preparations, see under ginger, p. 198. It is true that capsaicin (Thresh) is a phenolic compound, but the resinous pungent matter is not of this nature, hence the *raison d'être* of the test there given.

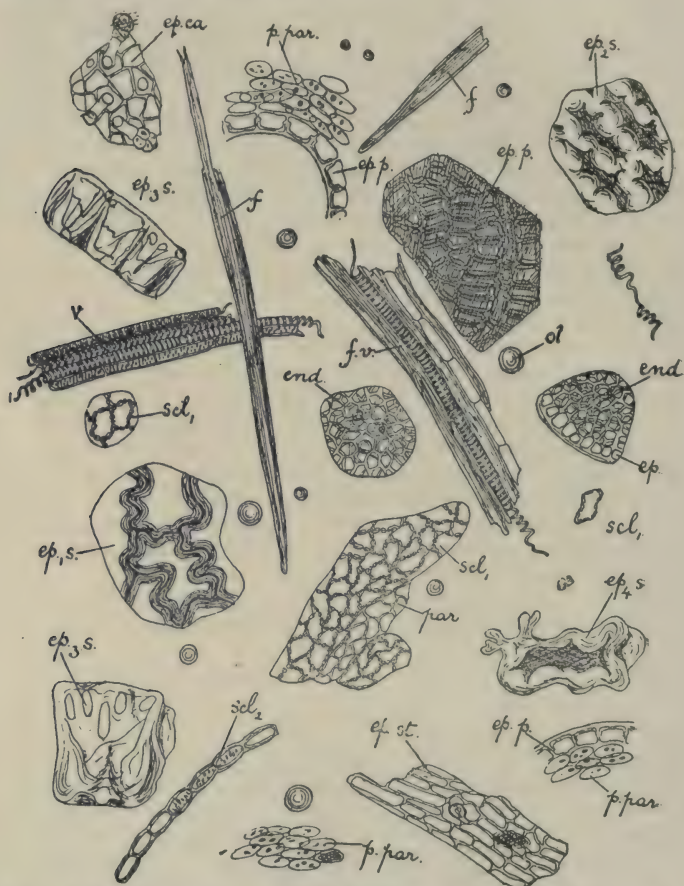


FIG. 24.—Powdered capsicum. *end.*, endosperm; *ep.*, epidermis of same; *ep. ca* upper epidermis of calyx; *ep. p.*, outer epidermis of pericarp; *ep. s.*, epidermis from flat surface of seed; *ep. s.*, epidermis from edge of seed; *ep. s.*, epidermis of seed, side view; *ep. s.*, isolated epidermal cell of seed coat; *f.*, sclerenchymatous fibres; *f.v.*, fibro-vascular bundle; *ep. st.*, epidermis of stalk; *o.*, oil; *par.*, parenchyma of inner epidermis of pericarp; *p. par.*, parenchyma of pericarp; *scl.*, sclerenchyma of inner epidermis of pericarp, seen from above; *scl.*, the same, side view $\times 108$. (Wallis.)

(By permission of the Editor of the "Pharmaceutical Journal".)

Closely allied to cayenne pepper is the so-called Paprika and also the spice known as Pimento.

Paprika is the ground fruit of *Capsicum annum* grown chiefly in Hungary, and has a mildly pungent taste, whilst Pimento, which is probably the product of an entirely different plant, is a nearly "tasteless" red pepper, produced in Spain and used for garnishing—such as for stuffing olives or giving a colour to various preserves, and also for feeding canaries on, in order to impart a characteristic orange colour to their plumage.

The principal adulterant of these "peppers" is olive or nut oil added principally to brighten their colours.

ANALYSES OF PAPRIKA (BROOKS).

	Moisture.	Volatile Ether Extract.	Non-volatile Ether Extract.	Starch.	Crude Fibre.	Albumeinoids. (N x 6.25).	Total Ash.	Water-soluble Ash.	Ash Insoluble in Hydrochloric Acid.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Hungarian (6 samples)									
Minimum (whole pods)	7.09	0.17	7.42	19.56	15.10	14.06	5.63	4.67	0.0
Maximum "	8.14	1.25	11.99	21.40	19.83	17.44	7.06	5.68	0.22
Spanish (2 samples)									
Minimum (whole pods)	7.10	1.18	10.39	16.52	15.37	14.62	5.24	4.59	0.05
Maximum "	—	—	—	—	—	16.87	6.79	5.79	0.06
Hungarian (7 samples)									
Minimum (shell alone)	9.01	0.44	4.01	21.16	16.66	12.50	5.50	4.85	0.03
Maximum "	9.76	1.10	6.69	24.52	23.61	15.37	6.90	6.10	0.08
Spanish (9 samples)									
Minimum (shell alone)	5.75	0.51	4.48	19.96	10.15	11.64	6.20	4.45	0.00
Maximum "	8.95	2.10	8.95	—	15.75	14.06	7.68	6.68	0.35
Hungarian (7 samples)									
Minimum (seeds and placenta)	4.05	0.95	17.66	17.36	17.29	16.56	3.06	1.72	0.05
Maximum "	4.56	1.90	22.34	18.16	20.11	21.19	4.93	3.72	0.09
Spanish (4 samples)									
Minimum (seeds and placenta)	3.63	1.56	18.99	16.12	19.48	15.50	3.41	2.23	0.04
Maximum "	4.33	2.25	19.80	—	24.01	16.25	5.20	4.35	0.11
Hungarian (7 samples)									
Minimum (stems)	2.83	0.27	1.38	—	19.86	14.37	10.03	6.93	0.30
Maximum "	8.80	0.78	2.39	—	29.94	18.00	12.25	9.28	0.61
Spanish (2 samples)									
Minimum (stems)	3.15	0.29	0.98	—	29.99	11.56	15.50	13.09	0.26
Maximum "	—	—	—	—	—	—	—	—	—

Added oil will be indicated by a high non-volatile ether extract.

MUSTARD.

The condiment mustard is the ground seed of *Sinapis (Brassica) alba*, or *Sinapis nigra* or preferably, a mixture of the two. On the continent, and in Asia, the seed of *Sinapis juncea*, the brown mustard, is also used. In pharmacy, both white and black mustard seeds are

official in the British Pharmacopœia, a mixture of the powdered seeds of the two plants being the official "*Sinapis*," used for the preparation of plasters.

The composition of the white and black mustard seeds is very similar, but important differences exist between the two.

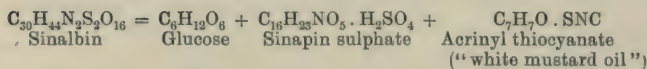
The pungency of mustard does not depend on the existence of any ready formed compound in the seed, but to the decomposition of glucosides in the presence of moisture by a ferment termed myrosin. This decomposition results in the formation of glucose, and a very pungent essential oil. Both seeds contain a fixed oil known as mustard oil, together with a considerable amount of albuminous matter and mucilage, but no starch. They each also contain sinapin thiocyanate $C_{16}H_{24}NO_5NS$, the white mustard containing about twice as much as the black mustard. Each contains the ferment myrosin, a soluble enzyme present in many of the cruciferous seeds. The most favourable temperature for its action on the glucosides is 45° to 50° C.; at 70° to 75° it is rendered inactive. The action is also inhibited by the presence of dilute hydrochloric acid.

The Glucosides of Mustard.—The glucoside contained in black or brown mustard seeds is sinigrin $C_{10}H_{16}NS_2KO_9 + H_2O$, or the potassium salt of myronic acid. That of white mustard is known as sinalbin $C_{30}H_{44}N_2S_2O_{15} + H_2O$. *Sinapis juncea* contains sinigrin or potassium myronate. Under the influence of the ferment myrosin, the glucosides split up in the following manner:—

BLACK MUSTARD.



WHITE MUSTARD.



The amount of myrosin present in the black seeds is not usually sufficient to convert all the glucoside present, whereas the white seeds contain more myrosin than is necessary to act on the whole of the glucoside present. Hence, although white mustard seed yields but a minute quantity of essential oil, black mustard will yield a higher amount of essential oil when mixed with white mustard. This explains the fact that the British Pharmacopœia describes white mustard as almost inodorous when treated with water, and black mustard as having a strong, pungent odour under the same circumstances, and directs a mixture of the two to be used as an official drug. The ground mustard of commerce is the farina separated from the husk by suitable sifting.

The following analyses by Piesse and Stansell ("*Analyst*," v. 161) represent the composition of white and black mustards. The essential oil, it is to be remembered, does not exist already formed in the seed,

so that the following figures represent the amount of essential oil formed in the mustard by hydrolysis:—

	White Mustard.					Black Mustard.			
	Whole Seeds.		Farina.			Whole Seeds.	Farina.		
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Moisture	9.32	8.0	—	5.78	6.06	8.52	4.35	4.52	5.63
Fatty oil	25.56	27.51	37.18	35.74	32.55	25.54	36.96	38.02	36.19
Cellulose	10.52	8.87	3.90	4.15	9.34	9.01	3.09	2.06	3.26
Sulphur	0.99	0.93	1.33	1.22	1.26	1.28	1.50	1.48	1.30
Nitrogen	4.54	4.49	5.05	4.89	4.25	4.38	4.94	5.01	4.31
Total proteids	28.37	28.06	31.56	30.56	26.56	26.50	29.81	30.25	26.06
Soluble albumen and myrosin	5.24	4.58	7.32	6.67	6.11	5.24	6.46	6.78	6.14
Volatile oil	0.06	0.08	0.03	0.04	0.03	0.47	1.44	1.50	1.38
Ash	4.57	4.70	4.22	4.31	4.30	4.98	5.04	4.84	4.91
H ₂ O soluble ash	0.55	0.75	0.44	0.55	0.33	1.11	1.01	0.98	0.77
Aqueous extract	27.38	26.29	36.31	36.60	33.90	24.22	31.64	32.78	31.41

The following may be taken as the limit values for genuine ground mustards:—

	Per cent
Moisture	5.0 " 9.5
Fatty oil (frequently part is extracted, which is regarded as a legitimate practice, when the amount will fall to 15 to 25 per cent)	32 to 39
Fibre (this may reach 10 per cent if much of the husk is ground with the farina)	1.8 " 5.5
Nitrogen	6.0 " 7.5
Ash	4.0 " 6.0
Ash insoluble in acid	0.08 " 0.5
Alcohol extract	19.0 " 25
" Starch " (diastase method)	0 " 2
" Starch " (by HCl Conversion—not true starch)	5 " 12

The ash of mustard seeds has the following composition according to the same chemists (the analyses were made on ash *not* free from organic matter):—

	White Seeds.	Black Seeds.
	Per cent	Per cent
Potash, K ₂ O	18.88 to 21.29	21.41
Soda, Na ₂ O	0.18 " 0.21	0.35
Lime, CaO	9.34 " 13.46	13.57
Magnesia MgO	8.17 " 10.49	10.04
Fe ₂ O ₃	1.03 " 1.18	1.06
SO ₃	7.06 " 7.16	5.56
Cl	0.11 " 0.12	0.15
P ₂ O ₅	32.74 " 35.00	37.20
Siliceous matter	2.92 " 3.07	2.79
Carbon	12.82 " 15.14	7.57

The Analysis of Mustard.—The following are the determinations necessary: (1) moisture, (2) ash, (3) fixed oil, (4) total sulphur.

A microscopic examination is necessary, and tests for special adulterants, the determination of the essential oil is often useful, and occasionally other determinations.

Moisture and Ash.—The moisture in a good quality mustard should not exceed 6 to 7 per cent; nor the ash from 4 to 6 per cent, of which from 0.3 to 1.2 is soluble in water. An ash of less than 4 per cent is an almost certain indication of the presence of an organic adulterant such as starch.

Fixed Oil.—Ten grms. of the mustard should be dried at 100° and extracted in a Soxhlet tube with ether. From 32 to 39 per cent should be obtained, a lower amount indicating the presence of an adulterant containing little or no oil.

Total Sulphur.—Genuine mustard contains from 1.1 per cent to 1.6 per cent of sulphur (usually about 1.3 per cent), as determined by heating the mustard with five times its weight of fuming nitric acid until completely oxidized and then precipitating the sulphates formed with barium chloride. Or the oxidation may be carried out by means of boiling with very strong alkaline permanganate of potassium.

The Detection of Adulterants.—Wheat flour or starch is added to mustard, sometimes with the idea of making it keep better. Such admixtures, however, must be disclosed or the sale of the mixed article constitutes an offence under the Food and Drugs Act. The detection of starch which would first be found by a microscopic examination, is simple, since mustard contains practically no starch. A gram of the sample should be boiled with water, and on cooling, a solution of iodine is added gradually, but not in too great excess. The production of a blue or blue-green colour is proof of the presence of added starch. The nature of the starch can only be decided by the results of the microscopic examination. If no adulterant but starch be present, its approximate amount may be deduced by the shortage in fixed oil, which averages 35 per cent in pure mustard. But as some of the mustard oil is often expressed before the mustard is prepared, this will not give necessarily reliable results. The most approximate method for the quantitative determination of the starch is to exhaust the sample first with ether and then with 60 per cent alcohol. The starch is now converted in the usual way by dilute acid, into sugar and estimated by reduction of Fehling's solution. Wheat flour may be taken as containing 72 per cent of starch.

Mineral adulterants are now rare, and are at once revealed by the ash determination. Turmeric is added, especially to mustards which have already been reduced with starch and so rendered too pale in colour to be attractive. The characteristic odour of turmeric is sufficient to prevent much being used, and in France it is regarded as a legitimate addition to mustard.

Turmeric is detected microscopically, and as it contains starch an iodine reaction is obtained, which can be well observed under the microscope. To verify the presence of this adulterant, 5 grms. should be extracted with methylated spirit and the extract concentrated to

about 1 c.c., and a piece of filter paper moistened with it and dried. The paper so prepared is treated with a few drops of concentrated boric acid solution, and dried. The characteristic reddish colour will result, which turns green to purple on moistening the spot with alkali.

It is said that the fluorescence of turmeric colouring matter enables one to detect a very small quantity. If 1 grm. be shaken for some time with warm castor oil and filtered, the oil will have a distinct green fluorescence if turmeric be present.

Martius yellow (dinitro-*a*-naphtholate of calcium) has been found by Waller and Martin ("Analyst," ix. 166). It is detected by shaking the mustard for several minutes with cold 95 per cent alcohol and filtering. The filtrate will be of a light yellow colour, and if it is evaporated to dryness and the residue is taken up with water, the aqueous solution will dye wool a light yellow colour. This, of course, is true of other yellow coal-tar dyes. The yellow solution is decolorized by hydrochloric acid, a yellow precipitate being formed.

Cayenne pepper has been found in mustard, especially in that which is adulterated with starch, as it is added to impart pungency to the diluted mustard. It can be detected by boiling 1 grm. of the mustard with alcohol and drying the extracted matter. The taste of the extract at once reveals the presence of capsicum, and if the residue be burned the acid fumes of capsicum cannot be mistaken. In foreign mustard, sold ready mixed as a condiment, sugar, tartaric acid, citric acid, turmeric, vinegar, wine, apple juice and a trace of sodium bisulphite are to be found.

Microscopic Examination.—Only a few fragments of the seed coats are to be found in ground mustard. Such as may be present will—in the case of white mustard—show some large hexagonal cells filled with stratified mucilage, the centre of each cell appearing as perforated by funnel-shaped tubes, which seem to terminate on the surface of the cell. When immersed in water these cells swell up enormously and then rupture. Numerous roundish or polygonal cells with thickened angles, or long palisade cells, are also characteristic of the seed coat. The mucilage cells in the seed coat of black mustard is scarcely stratified at all and is therefore less conspicuous. Nor do they swell so much in water. The sample should be defatted, and the main portion will be found to consist of the fragments of the seeds proper. Numerous cells will be found containing aleurone grains which stain yellow with picric acid; no starch grains are to be found. The bulk of the powder will be found to consist of small masses of delicate parenchymatous cells.

Essential Oil of Mustard.—The essential oil of mustard of commerce is obtained by distilling the seeds of the black mustard.

It is official in the British Pharmacopœia, which authority requires it to have a specific gravity 1.018 to 1.030. It should distil between 147° and 152°, and the first and last distillates should have the same specific gravity as the original oil. In the formation of the essential oil by the hydrolysis of the glucoside, the chief product of the reaction is allyl iso-thiocyanate (allyl thiocarbimide), a pungent and disagree-

able liquid. A small quantity of the normal allyl thiocyanate is also formed, together with traces of cyanallyl and carbon disulphide. The oil is a pungent and unpleasantly smelling liquid of specific gravity 1.015 to 1.030, and optically inactive. It boils almost completely between 148° and 155° and has a refractive index from 1.526 to 1.530. As mustard oil consists almost entirely of allyl iso-thiocyanate, and

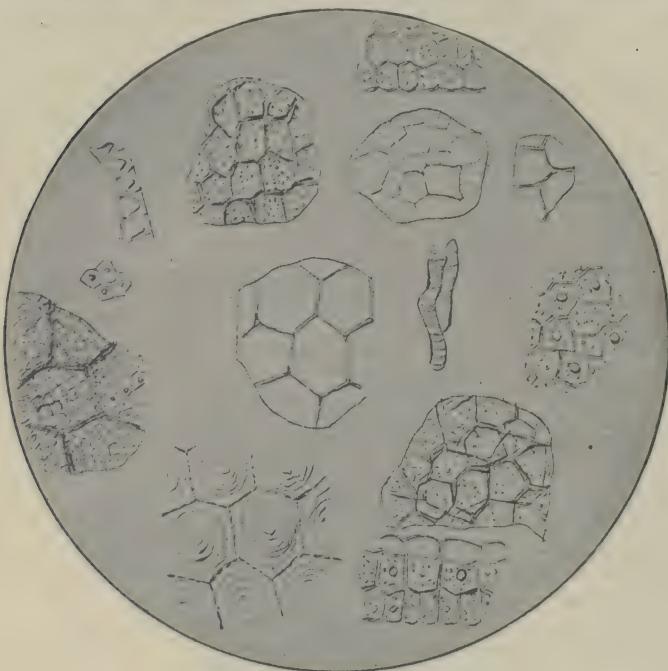
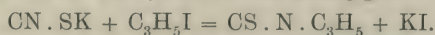


FIG. 25.—Powdered mustard.

the latter body is easily prepared artificially, there is an artificial oil on the market. This is made by distilling allyl iodide or bromide with alcoholic solution of potassium thiocyanate—a molecular rearrangement to the iso-thiocyanic radicle taking place. Thus—



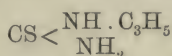
Pure allyl iso-thiocyanate is a liquid of specific gravity 1.017 at 10°, boiling at 151°.

The artificial oil, however, is not far different in price from the natural oil, and is not official in any pharmacopœia. The amount of allyl iso-thiocyanate present in the oil can be approximately estimated by heating a known quantity with an alcoholic solution of ammonia, when allyl-thio-urea is formed.

For the determination of the amount of allyl iso-thiocyanate present, which should not be less than 92.5 per cent in any oil; or of the

amount of mustard oil in spirituous preparations, or in the mustard itself one of the following processes should be used:—

Three grms. of the oil and 3 grms. of alcohol are shaken in a flask with 6 grms. of a 10 per cent solution of ammonia. It should become clear after standing for a few hours, or rapidly if warmed to 50° C., and deposit crystals of allyl-thio-urea (thiosinamine).



To determine the quantity, decant the mother liquor and evaporate it slowly on the water bath in a tared capsule, adding fresh portions slowly as the smell of ammonia disappears. Then add the crystals from the flask to those in the capsule, rinsing the flask with a little alcohol; and heat the capsule on the water bath to a constant weight. Three grms. of oil should yield between 3.25 and 3.5 grms. of thiosinamine, which should melt at 70° to 74°. One hundred and sixteen parts of thiosinamine, correspond to 99 parts of allyl iso-thiocyanate. Gadamer (*"Arch. Pharm."* 1899, pp. 110, 237) recommends the following process. The mustard oil is dissolved in alcohol to form an exactly 2 per cent solution. Five c.c. (4.2 grms.) of this solution are allowed to remain with 25 c.c. of decinormal solution of silver nitrate and 5 c.c. of ammonia for twenty-four hours in a well-stoppered 50 c.c. flask. It is then made up to 50 c.c. with water and filtered from the precipitated silver sulphide; 25 c.c. of the filtrate are mixed with 4 c.c. of nitric acid and a few drops of ferric sulphate solution, and titrated with decinormal ammonium thiocyanate solution, until the characteristic red colour of the ferric thiocyanate appears. From 4.1 to 4.5 c.c. of the solution (corresponding to 1.85 to 2.0 per cent of allyl thiocyanate in the alcoholic solution) should be required.

Grützner converts the thiocyanate into thiosinamine, which he oxidizes with peroxide of sodium, and weighs the resultant sulphuric acid as barium-sulphate. From the figures obtained in his analyses, Grützner concludes that a mustard oil containing 28.60 per cent of sulphur (equivalent to 88.48 per cent of iso-thiocyanate) may be regarded as pure. P. Roeser proposes to modify Gadamer's method for the determination of the sulphur content of oil of mustard so as to determine the excess of silver nitrate in an ammoniacal solution, instead of an acid solution according to Volhard's method, as is usually done. Roeser operates in the following manner: when the conversion of the thiosinamine with silver nitrate, after twenty-four hours standing, has taken place, an excess of one-tenth normal solution of potassium cyanide is added to 50 c.c. of the clear filtrate, and the excess of potassium cyanide titrated back with one-tenth normal solution of silver nitrate, in the presence of a few drops of a weak ammoniacal solution (5 per cent) of potassium iodide. Schimmel & Co. prefer the following method. About 5 grms. of a solution of 1 gm. mustard oil in 49 grms. alcohol are mixed in a measuring flask of 100 c.c. capacity with 50 c.c. decinormal solution of silver nitrate and 10 c.c. of ammonia (d_{15}° 0.960); the flask is then closed, and with frequent agitation left standing for twenty-four hours with the light

excluded. The flask is then placed for half an hour in water at 80°, during which time it is again repeatedly shaken, then cooled down to the temperature of the room, filled up with water to the mark, shaken up, and filtered. Fifty c.c. of the filtrate are titrated with one-tenth normal solution of ammonium thiocyanate, after adding 6 c.c. nitric acid (d_{15}° 1.153) and a small quantity of solution of iron alum, until a change of colour from white to red takes place. In order to ascertain the whole quantity of silver solution which has entered into reaction, the number of c.c. of ammonium thiocyanate solution used is doubled, and the product subtracted from 50. The percentage of allyl iso-thiocyanate in the mustard oil is obtained by means of the following formula:—

$$\text{CSNC}_3\text{H}_5 = \frac{24.7875 \times a}{b}$$

A = number of c.c. of decinormal solution of silver nitrate used, b = spirit of mustard used, in grammes. Mustard oil determinations carried out by them in the manner described, showed in the case of natural oil a content of about 94 per cent allyl iso-thiocyanate, whilst in artificial oil about 98 per cent was found.

In the case of the mustard itself Förster recommends the following process ("Journ. Chem. Soc." 54, 1350):—

Twenty-five grms. of the powder is made into a thin paste with water and allowed to stand for an hour, and then the essential oil distilled by steam through a condenser into a 250 c.c. flask, containing 50 c.c. of alcohol saturated with ammonia, the end of the condenser dipping under the surface of the fluid.

When about 150 c.c. has distilled, the flask is allowed to stand for twelve hours, and then heated to boiling-point, and freshly prepared mercuric oxide (made by decomposing a 5 per cent solution of mercuric chloride with caustic potash and boiling the mixture) is added, to combine with all the sulphur present. The mixture is again boiled and a little potassium cyanide added before it is quite cold. The mercuric sulphide is collected on a tared filter, dried and weighed. The amount of HgS multiplied by 0.4266 gives the amount of mustard oil present. Schlicht's process is very satisfactory also. He proposes ("Zeit. Anal. Chem." xxx. 661) to distil the mustard oil from the mustard in a current of steam and then proceeds as follows:—

To the aqueous distillate containing the mustard oil are added 20 parts of potassium permanganate and 5 parts of caustic potash or caustic soda (which reagents must be free from sulphate) for each part of mustard oil supposed to be present. The mixture is shaken for some time in a closed flask, and finally heated nearly to boiling. The whole of the sulphur is thus oxidized to sulphuric acid. After cooling the solution somewhat, 5 c.c. of alcohol should be added for every grm. of permanganate previously used. The whole of the manganese is thus precipitated. The mixture is then completely cooled, largely diluted, made up to a known volume, and filtered. A measured portion of the filtrate is slightly acidified with hydrochloric acid, and treated with a solution of iodine in potassium iodide until a feeble

yellow colour remains even after warming. This reoxidizes any sulphurous acid which may have been produced by reduction by means of aldehyde, and also removes the aldehyde itself.¹ The sulphuric acid is now determined by precipitation with barium chloride, and the weight of barium sulphate multiplied by 0.42492. The product gives the amount of mustard oil.

Piesse and Stansell determine the essential oil formed in mustard from the glucosides present, in the following manner, which according to Sutton forms an approximate method for the estimation of the proportion of brown mustard in a mixture of the two kinds.

Twenty-five grms. of the crushed brown seeds are mixed with about 6 grms. of crushed white seeds, and 300 c.c. of cold water added. The mixture is allowed to stand in a 700 c.c. flask for five to six hours at ordinary temperature. The contents of the flask are then distilled and the distillate collected in a flask containing 30 c.c. of strong ammonia. Usually about 50 c.c. of distillate are collected, but distillation should proceed till no more oil drops are carried over. When combination is complete, the distillate is evaporated and the thiosinamine dried at 100° and weighed. The weight $\times 0.853$ gives the amount of mustard oil—or if multiplied by 3.578 the amount of potassium myronate from which it was derived. As the average amount of potassium myronate is fairly constant in brown mustard seeds—about 5.15 per cent, yielding 1.33 per cent of thiosinamine so that the weight of the latter multiplied by 75 will give the approximate amount of brown mustard present in the sample.

Jorgensen recommends ("Analyst," xxxiv. 489) the estimation of the amount of nitrogen in the "thiosinamine" obtained by distilling the essential oil into a solution of ammonia and evaporating the solution. He states that the following percentages of nitrogen are obtained from the oils of various species of brassica:—

	Per cent
Brassica (sinapis) nigra	24.14
" dichotoma	20.52
" glauca	20.34
" ramosa	18.36
" napus	21.21
" rapa	20.33

He recommends adding a little powdered white mustard to provide the ferment and then distilling the essential oil.

White mustard seeds, from *Sinapis alba*, contain the glucoside sinalbin, $C_{30}H_{44}N_2S_2O_{16}$, which on decomposition in the same manner as the glucoside of black mustard, yields glucose, sinapine sulphate, and the evil-smelling oil, acrinyl-isothiocyanate (*p*-hydroxy-benzyl-isothiocyanate).

Acrinyl iso-thiocyanate, or "white mustard oil," is a yellowish oily liquid, of pungent odour and unpleasant hot taste. It is prepared synthetically by treating *p*-hydroxy-benzylamine with carbon disulphide, and the resulting compound with mercuric chloride.

¹ The reduction of sulphuric acid in dilute alkaline solution by aldehyde is highly improbable. Addition of bromine-water would do instantaneously and certainly what Schlicht effects by iodized potassium iodide.

Fixed Oil of Mustard.—If it is considered necessary to examine the fixed oil of mustard, it should have the following characters :—

	Sinapis Nigra.	Sinapis Alba.	Sinapis Juncea.
Specific gravity at 15°	0.915 to 0.920	0.9125 to 0.916	0.915 to 0.922
Saponification value	173 „ 176	170 „ 175	172 „ 182
Iodine value	96 „ 106	92 „ 98	102 „ 110
Refractive index at 15°	1.4672	1.4735	1.4699
Melting-point of fatty acids	15° to 17°	15° to 16°	15° to 17°
Molecular weight of „ „	300	302	296

CLOVES.

Cloves are the dried flower buds of *Eugenia caryophyllata*, and are almost invariably sold whole, and are largely used as a spice. They are official under the name “Caryophyllum” in the British Pharmacopœia, which describes them as follows:—

“About $\frac{5}{8}$ ths of an inch long, each consisting of a dark brown, wrinkled, subcylindrical, somewhat angular calyx tube, which tapers below and is surmounted by four thick, rigid, patent teeth, between which are four paler imbricated petals enclosing numerous stamens and a single style. Odour strong, fragrant, and spicy; taste very pungent and aromatic. Cloves should emit oil when indented with the finger-nail. Incinerated they should not yield more than 7 per cent of ash.”

As cloves are particularly characteristic in appearance, and are almost invariably sold whole, adulteration is not common. Admixture with the fruits of the clove (“mother cloves”) has been observed, but this is not now practised. The fruit has the shape of a very small olive, and is crowned with the four teeth of the calyx and the remains of the style. Clove stalks are also mixed with the clove buds at times. These are distinguished by the absence of the stamens, style, etc., which renders their detection quite simple by the naked eye. Apart from such admixture, the only other cases noticed by the author are the addition of partially spent cloves, from which a large amount of the essential oil is distilled, and the admixture with water-damaged cloves. In the former case, it is a common thing for some distillers to dry the cloves from which they have distilled the greater part of the essential oil, and sell them to second-rate spice dealers. This fraud is practised to a much larger extent than would be expected. The author is personally acquainted with a distillery which puts about 40 tons of such partially spent cloves on the market per annum. The greater part of these find their way, however, to the continent. In the case of water-damaged cloves, but little exception is to be taken as it is little more than a matter of appearance. It is, however, often a matter of importance to decide whether such damaged cloves have been exposed to river or sea water, since the conditions of insurance policies differentiate between the two. The sea-damaged cloves, on soaking for a

short time in distilled water, give a copious precipitate with silver nitrate.

Cloves contain from 12 to 20 per cent of essential oil, rarely below 15 per cent. Clove stems, however, contain only about 6 per cent.

The following represent the average compositions of cloves and of clove stems:—

Observers.	Water.	Ash.	Essential Oil.	Fixed Ether Extract.	Fibre.	Nitrogen.	Tannin.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Parry	8.6	12.4	16.5	8.8	10.1	0.9	17.5
Richardson	2.9 to 10.7	5.5 to 13	10.2 to 18.9	7.1 to 10.2	6.2 to 9.7	0.76 to 1.12	11.7 to 22.1
McGill	5 „ 11.8	5 „ 7.0	9.2 „ 19.6	0.9 „ 10.2	—	—	—

Winton, Ogden and Mitchell ("Conn. Exp. Sta. Rep." 1898, 206), give the following fuller analyses of eight samples of genuine cloves:—

	H ₂ O	Ash.			Ether Extract.		Alcohol Extract.
		Total.	HCl. Sol.	Insol. in HCl.	Volatile.	Non-volatile.	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Maximum	8.26	6.22	3.75	0.13	20.53	6.67	15.58
Minimum	7.03	5.28	3.25	0.00	17.82	6.24	13.99
Mean	7.81	5.92	3.58	0.06	19.18	6.49	14.87
Stems	8.74	7.99	4.26	0.60	5.00	3.83	6.79

	"Starch" by HCl Conversion.	Starch by Diastase Method.	Fibre.	Nitrogen.	Tannic Acid.
	Per cent	Per cent	Per cent	Per cent	Per cent
Maximum	9.63	3.15	9.02	1.13	20.54
Minimum	8.19	2.08	7.06	0.94	16.25
Mean	8.99	2.74	8.10	0.99	18.19
Stems	14.13	2.17	18.71	0.94	18.79

Mineral Matter.—Any excess over 7 per cent of ash is due either to adulteration or to the presence of too much dirt. The average figures are from 5.5 per cent to 6.5 per cent, of which 60 per cent is soluble in water; the ash insoluble in HCl should not exceed 0.1 per cent. The ash of clove stems is almost 8 per cent so that a considerable proportion of stems might be present without the ash limit being exceeded.

Extractives.—The ether extract, after driving off the essential oil

will always fall between the values 5.5 per cent and 7 per cent in normal cloves. The alcoholic extract, similarly dried varies from 14 to 16 per cent.

Tannin.—Cloves contain much tannic acid. If this be determined as described on page 192, it should not be materially less than 16 per cent (this is probably a modification of ordinary tannic acid).

The Essential Oil.—This can only be determined properly on a large sample, with a proper experimental still. At least 1000 grms. should be exhausted by steam distillation and the essential oil collected and measured. Its specific gravity may be taken as 1.050. Any result lower than 15 per cent is strongly indicative of the presence of partially exhausted cloves.

Cripps and Brown ("Analyst," xxxiv. 518) recommend the determination of the essential oil in this and other spices, by first estimating the total amount of volatile matter, and then determining the moisture by the amount of acetylene liberated from calcium carbide, returning the difference as essential oil.

They use 0.5 grm. of the spice in fine powder, in a stout tube 5 in. long and $\frac{5}{8}$ in. in diameter. Dried sand is added to the depth of about $\frac{3}{4}$ in. and then calcium carbide to within $1\frac{1}{4}$ in. of the mouth of the tube. This is connected with a calcium chloride tube to prevent moisture from outside reaching the carbide, and then with a nitrometer tube in strong brine. The tube with the spice, etc., is immersed in a brine bath and heat is applied until no increase in the volume of the gas in the nitrometer takes place in five minutes. The gas is then measured after adjusting the temperature and pressure, and the number of c.c. multiplied by 0.001725 gives the weight of water in grammes in the amount of the sample used. In this way the above-named chemists found the following amounts of essential oil in samples known to be pure:—

Cloves	12.75	to 17.90
Allspice	1.64	" 3.67
Caraway	2.49	" 5.24
Whole mace	6.25	" 10.80
Ground mace	2.86(?)	" 7.15
Ginger	2.24	" 3.48
Fennel	1.97	" 4.00

The presence of exhausted cloves, then, is indicated by a low essential oil yield, a low soluble ash, and a low tannin content.

Microscopic Examination.—Under the microscope cloves in coarse powder will show numerous large oil cavities—often broken in the powdering, fibrovascular bundles embedded in parenchymatous tissue, with spiral and other vessels. Crystals of calcium oxalate are plentiful but no, or practically no, starch can be found. The presence of exhausted cloves will be indicated by the disrupted nature of the tissues. Clove stems are easily detected by the presence of well-marked "stone cells," very thick-walled large cells, penetrated by radial pores (see illustration). Starch is also to be found in small quantity. "Mother cloves" or fruit, show similar "stone" cells, except that they are usually much larger—often ten times as long as they are wide.

Oil of Cloves.—Essential oil of cloves is largely used in flavouring and is usually sold, diluted with alcohol, under the name of "essence of cloves". For its examination the alcohol should be removed in a current of warm air, and the oil then examined. Oil of cloves is official in the British Pharmacopœia, that authority requiring it to have a specific gravity not below 1.050.

The principal constituent of clove oil is eugenol, besides which there

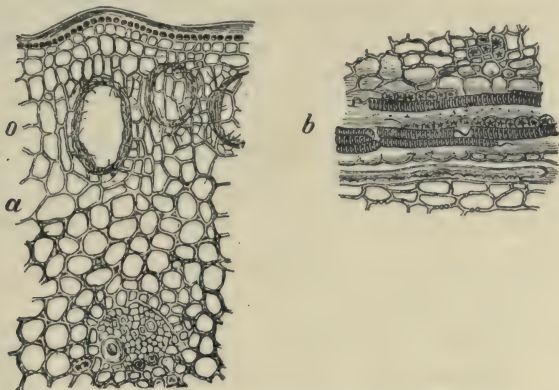


FIG. 26.—Section of clove buds. *o*, oilreceptacles; *a*, parenchyma; *b*, spiral vessels.

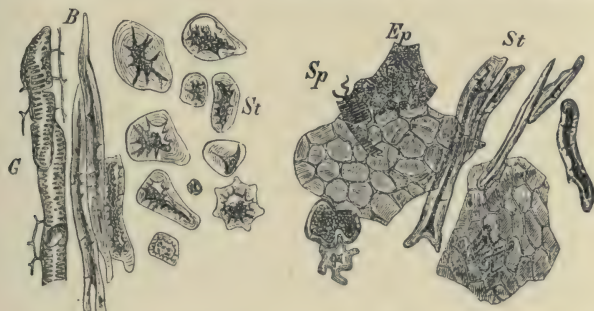


FIG. 27A.—Clove stems.

FIG. 27B.—Mother cloves.

G, scalariform vessels; *B*, thickened fibres; *St*, stone cells; *Ep*, epidermis.

are present, the sesquiterpene caryophyllene, esters of eugenol, methyl alcohol, furfural, amyl-methyl-ketone and traces of other bodies. Pure clove oil should have the following characters:—

	Per cent
Specific gravity	1.048 to 1.066
Refractive index at 20°	1.5280 „ 1.5320
Optical rotation	– 0°20' „ – 1°35'
Eugenol (by absorption)	80 at least, usually 85 to 93
„ (Thom's method)	76 to 88

The method for the determination of the eugenol suggested by
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Umney gives useful approximate results, but is subject to a not inconsiderable error. This consists in shaking a known weight of the oil with a 10 per cent aqueous solution of potassium hydroxide in a Hirschsohn flask, and allowing the unabsorbed portion to rise into the graduated neck. This is measured and its volume corrected by multiplying it by '908—the specific gravity of the sesquiterpene—and the unabsorbed portion returned as caryophyllene, the difference being reckoned as eugenol. The globules of uncombined hydrocarbons have a great tendency to stick round the top of the flask and require some "coaxing" to rise and agglomerate in the neck of the flask. Heat will accelerate and assist this however. But the source of error lies in the fact that the aqueous solution of potash and potassium eugenate dissolves some of the sesquiterpene, which is thus reckoned as eugenol, and a too high result is obtained. The process proposed by Thom, although more tedious, gives more exact results. This depends on the conversion of the eugenol into benzoyl-eugenol. The following are the details, which should be carefully observed in order to secure accurate results:—

Five grms. of the oil are heated on a water bath with 20 c.c. of a 15 per cent solution of caustic soda for thirty minutes.

After allowing the hydrocarbons to separate, the eugenol soda solution is run off, and the hydrocarbons washed with dilute soda solution twice, the washings being added to the original soda solution. The reaction is now effected at water-bath temperature with 6 grms. of benzoyl chloride. The whole is allowed to cool, and the crystalline mass is transferred to a beaker with 55 c.c. of water. It is heated in order to melt the crystals, and well agitated with the water to wash the benzoyl eugenol. This washing is repeated twice. The crystalline mass is then transferred to a beaker with 25 c.c. of 90 per cent alcohol, and warmed till complete solution takes place. The solution is allowed to stand till the bulk of the crystals have separated out, and is cooled to 17° and filtered through a paper 9 centimetres in diameter, previously dried and tared. The filtrate measures about 20 c.c. and the crystals are washed with more alcohol until it measures 25 c.c. The paper and crystals are then dried in a weighing glass and weighed, the temperature of drying being not more than 101° C. The solubility allowance for 25 c.c. of alcohol is 0.55 gm. The total eugenol is calculated from the formula.

$$P = 4100 \frac{a + 0.55}{67b}$$

where P is the percentage, *a* the weight of benzoyl-eugenol obtained, and *b* is the weight of oil of cloves used.

Verley and Bolsing propose the following method: It depends on the fact that acetic and other anhydrides react with phenols in excess of pyridine. Eugenol reacts readily forming eugenyl acetate and acetic acid, the latter combining with pyridine to form pyridine acetate. This compound reacts towards indicators such as phenolphthalein in the same way as acetic acid, and therefore a titration is possible.

Verley and Bolsing use from 1 to 2 grms. of the oil, which is placed in a 200 c.c. flask, and 25 c.c. of a mixture of acetic anhydride (15 parts) and pyridine (100 parts). The mixture is heated for thirty minutes on a water bath, the liquid cooled, and 25 c.c. of water added. The mixture is well-shaken and titrated with normal potash, using phenolphthalein as indicator. A blank experiment is carried out without the eugenol, and the difference between the titration figures in c.c. of normal alkali, multiplied by 0.582, gives the amount of eugenol in the sample taken.

ALLSPICE.

Allspice or Pimento is the dried, fully grown unripe fruit of *Pimenta officinalis*, and as such is official in the British Pharmacopœia.

The fruits consist of dark reddish-brown, nearly globular two-celled fruits, about 5 to 8 millimetres in diameter. The pericarp is rough and brittle and covered by the remains of a four-toothed calyx in the form of a raised ring, surrounding the remains of the style. Each cell contains a single brownish-black reniform seed. Allspice owes its characteristic flavour to from 3 to 6.5 per cent of an essential oil. The spice is generally sold whole, and the author has never met with an adulterated sample. The genuine spice has the following characters:—

	Per cent	
Total ash	4.0 to	5.5
Ash soluble in water	at least 50 of that	
Ash insoluble in HCl	up to	0.2
Alcoholic extract	10 to	13
Fixed ether extract	4.0 "	6.2
Essential oil	3.0 "	6.5
Fibre	13 "	19
Nitrogen	0.65 "	0.90
Tannin	8 "	13

Microscopic Examination.—Under the microscope powdered allspice is characterised by stone cells, similar to those in cinnamon, etc., a number of large oval cells of a port wine colour, parenchymatous cells containing many small starch cells; and cells containing essential oil. Foreign starch grains are easily detectable.

Essential Oil of Pimento.—The principal constituent of the essential oil is eugenol, the remainder consisting chiefly of a sesquiterpene.

The specific gravity of the oil is, to an extent, an indication of the amount of eugenol present, and should vary between 1.040 and 1.055. The British Pharmacopœia, in which this oil is official, states that the specific gravity should not fall below 1.040. The oil is laevorotatory, but never exceeds -4° , usually about -2° . It is easily soluble in 90 per cent alcohol and in twice its volume of 70 per cent alcohol. Eugenol boils at 247° , consequently the fraction 245° to 250° should be considerable—in genuine oils not below 60 per cent, usually 70 per cent or over. The amount of eugenol, as estimated by Thom's

process (see Oil of Cloves), should not be less than 65 per cent; and the residue not absorbed by caustic potash solution should not exceed 25 per cent.

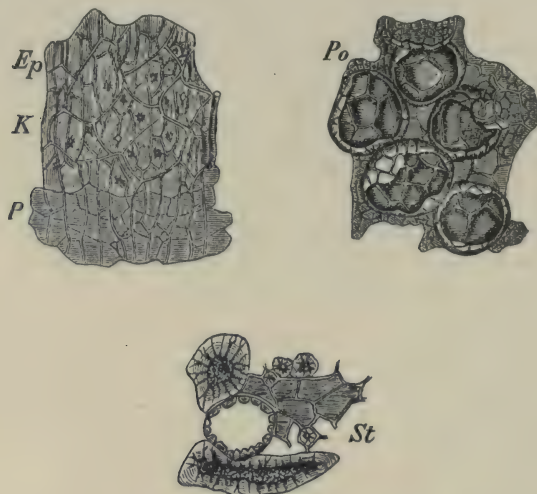


FIG. 28.—Powdered allspice. *Ep*, epidermal cells; *P*, brown parenchyma; *K*, crystals; *Po*, oil cells; *St*, stone cells.

CINNAMON.

This spice is the dried inner bark of shoots from the truncated stocks of *Cinnamomum zeylanicum*, and is official in the British Pharmacopœia. It is usually sold in quills (the dried rolled bark of the shoots) and is only adulterated in the form of powder. Cinnamon bark yields from 0·5 per cent to 1 per cent of an essential oil which is largely used for flavouring purposes.

The following are the characters of genuine cinnamon:—

	Per cent	
Total ash	3·5 to 5·5	rarely up to 6
Ash soluble in water	1·6 „ 2·4	
„ insoluble in HCl	usually under 0·5	
Fibre	25 to 33	
Alcoholic extract	10 „ 15	
Nitrogen	0·5 „ 0·65	
Fixed ether extract	1·4 „ 1·7	
Essential oil	0·8 „ 1·30	

The following analyses are those of Winton, Ogden and Mitchell:—

	Per cent
Moisture	7.79 to 10.48
Total ash	4.16 „ 5.99
Ash soluble in H ₂ O	1.40 „ 2.71
„ insoluble in HCl	0.02 „ 0.58
Volatile ether extract	0.72 „ 1.62
Non-volatile „	1.35 „ 1.68
Alcohol extract	9.97 „ 13.60
“Starch” by acid conversion	16.65 „ 22.0
Fibre	34.38 „ 38.48
Nitrogen	0.52 „ 0.65

The powdered bark of *Cinnamomum cassia*, the Chinese cassia tree, is sometimes used as an adulterant, but can only be detected by its odour if present in large quantity, or microscopically. The powder should be fine enough to pass through a very fine sieve, and should be bleached by immersion in a solution of chlorinated soda. If a specimen so bleached be examined in glycerine, the characteristic thick-walled



FIG. 29.—Powdered cinnamon bark.

sclerenchymatous cells with radial markings, ordinary parenchymatous cells, many containing crystals of calcium oxalate (which appear well marked under a polarizer) and bark fibres will be seen. A preparation stained with a hot solution of soudan red shows up the characteristic “secretion cells,” elongated cells with suberized walls, which take the

stain very deeply. The starch should be examined, and compared with that of a standard preparation, and also with that of cassia bark. The characteristic differences between cinnamon and cassia are that in the latter the starch cells are rather larger, and the bark fibres are stouter. But only a comparison with type samples will render these differences useful. It has been pointed out by Greenish that the differences between the two barks become slighter as one compares the lower-grade cinnamons with the best-grade cassias.

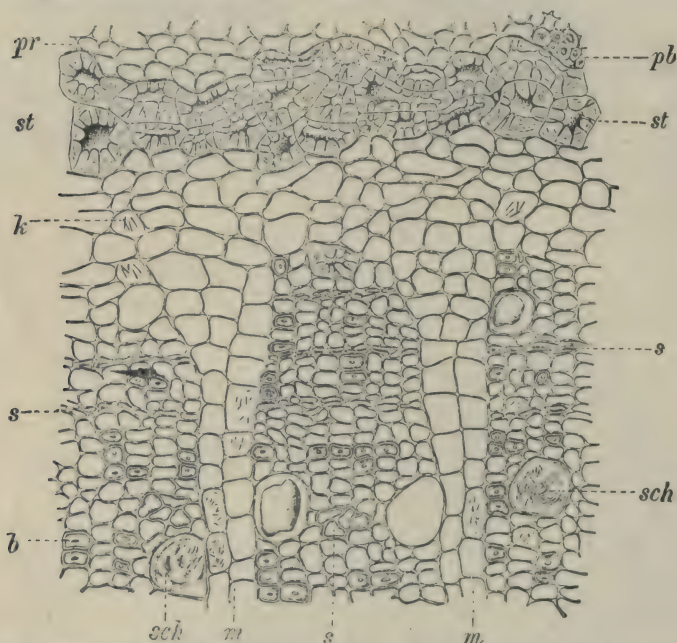


FIG. 90.—Cinnamon bark, transverse section. *b*, bast fibres; *k*, crystals of calcium oxalate; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma; *s*, sieve tubes; *sch*, secretion cells; *st*, sclerenchymatous cells, forming an uninterrupted ring $\times 160$. (Moeller.)

Essential Oil of Cinnamon.—This is understood to be the oil distilled from the bark, although nearly every part of the plant yields an essential oil. It is distilled in Europe, but a good deal is prepared in Ceylon and exported westwards.

The majority of that exported, however, is not genuine. Either the leaves are added to the bark when distilled, or cinnamon leaf oil is added to the oil after distillation. The important difference between the two oils is that the bark oil owes its characteristic odour to the cinnamic aldehyde it contains, whilst the leaf oil contains only traces of that body; the chief constituent of the latter oil is eugenol, the characteristic phenol of the oils of cloves and pimento.

The pure bark oil has a specific gravity of 0.998 to 1.038.

Adulteration with the leaf oil or with clove oil increases this figure. The oil is optically inactive, or at most lævorotatory to the extent of -1° . The ascertained constituents are the terpene phellandrene, cinnamic aldehyde, and eugenol. The British Pharmacopœia, in which this oil is official, gives the following limits: specific gravity, 1.025 to 1.035; cinnamic aldehyde at least 50 per cent; should not yield a decided blue coloration with ferric chloride solution. It is

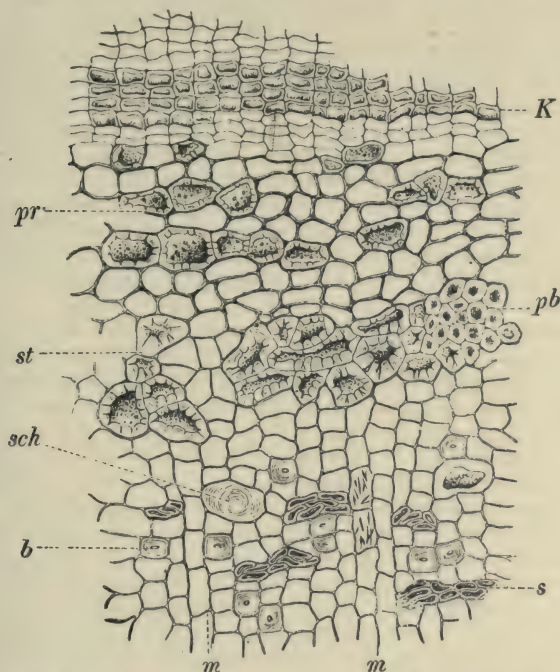


FIG. 31.—Cassia bark, transverse section. *b*, bast fibres; *K*, sclerenchymatous cork cells; *m*, medullary rays; *pb*, primary bast fibres (pericyclic fibres); *pr*, cortical parenchyma, with sclerenchymatous cells; *s*, sieve tubes; *sch*, secretion cell; *st*, sclerenchymatous cells, forming an interrupted ring $\times 160$. (Möller.)

very rare, however, to find an oil with only 50 per cent of aldehyde present. Adulteration with much leaf oil causes the characteristic blue colour given by eugenol to be developed when a few drops of a solution of ferric chloride are added to a solution of the oil in alcohol. The amount of eugenol estimated as described under oil of cloves, should not exceed 8 per cent. More than this indicates the presence of leaf oil.

The most important method of examination is the determination of the percentage of cinnamic aldehyde. In this process the following details should be observed. Ten c.c. of the oil are run into a Hirschsohn flask (capacity about 100 to 150 c.c., with a neck about 5 inches

long and $\frac{1}{4}$ inch in diameter, graduated in $\frac{1}{10}$ th c.c.). The flask is then filled about $\frac{3}{4}$ full with a 30 per cent solution of sodium bisulphite, and the whole well shaken. The flask is then placed on the water bath for several hours with occasional shaking, until the precipitated compound of the aldehyde and bisulphite is completely dissolved, and only a clear oil floats on the surface. Bisulphite solution is then carefully poured in until the oil is driven up into the neck, and when it has attained the temperature at which the oil was measured, the amount is read off. This gives the percentage of non-aldehydic constituents, the difference being returned as cinnamic aldehyde. Pure oils should not give less than 60 per cent of aldehyde, the best oils yielding 65 to 70 per cent, or occasionally even higher. Oils with much higher cinnamic aldehyde value usually contain the synthetic aldehyde. Strictly speaking, these percentages are by volume, but the errors of reading the result, and those due to solubility of the non-aldehydes in the aqueous liquid render any correction for the specific gravity of the constituents unnecessary in practice. Care must be taken that every particle of the aldehyde compound is dissolved, as otherwise the reading of the oily layer will be obscured, and a serious error may be introduced.

Hanus has recently published a new method for the determination of cinnamic aldehyde in cassia and cinnamon oils ("Pharm. Central." 1904, 37) depending on the combination of the aldehyde with semi-oxamazide. Ten grms. of finely powdered hydrazine sulphate are dissolved in a solution of 9 grms. of caustic soda in 100 c.c. of water and the alkaline sulphate produced is precipitated by the addition of 100 c.c. of alcohol. After filtration the solution is warmed, 9 grms. of oxamethane are added in small portions, the whole warmed for half an hour and allowed to cool. The azide separates in crystalline tables and these are separated and recrystallized. To estimate the aldehyde by means of this reagent, a small quantity, not more than 0.2 gm. of the oil is well shaken in 85 c.c. of water, and about 0.35 gm. of semi-oxamazide in 15 c.c. of hot water is added and the whole well shaken. After five or ten minutes the compound begins to be precipitated, and after standing twenty-four hours can be collected on a Gooch filter, washed with cold water, and dried for a few minutes at 105°. The amount of the precipitate is multiplied by 0.6083 to obtain the amount of aldehyde. The constitution of the semi-oxamazone of cinnamic aldehyde is $\text{NH}_2 \cdot \text{CO} \cdot \text{CO} \cdot \text{NH} \cdot \text{N} : \text{CH} \cdot \text{CH} : \text{CH} \cdot \text{C}_6\text{H}_5$.

NUTMEGS.

As, in the case of mace, the nutmeg of commerce is derived from *Myristica fragrans*, of which it is the dried seed, divested of its testa. As a rule nutmegs are sold in the whole condition, powdered nutmegs being rarely seen in retail shops. They are rarely adulterated except (1) by admixture with other species of nutmegs, (2) when worm-eaten nuts accumulate, the holes are sometimes filled with extraneous matter and the nut is coated with lime, (3) by the addition of partially exhausted nuts.

(1) The genuine nutmeg can be distinguished from the usual admixture the so-called "long" nutmeg, or Macassar nutmeg (*M. argentea*) by its appearance. The true nutmeg resembles an olive in shape, whereas the Macassar nutmeg more closely resembles an enlarged date stone. The latter are far less fragrant than the former.

(2) As some organic powder is used for this purpose, and that only to a minute extent—since the filling up of the tiny holes is done with a view of passing off inferior nuts as of better quality, this can hardly be detected except by carefully cutting and probing the nutmeg. The custom of liming nutmegs originated for the purpose of perservation from the attacks of insects; to-day it is often practised solely for the purpose of concealing the inferior quality of low-grade nutmegs.

(3) Occasionally small holes are drilled in nutmegs and some of the fat extracted by soaking in hot water, or some of the essential oil driven off. The holes are then filled and the nutmegs limed as before. This can only be detected by a physical examination, and a determination of the amount of fat present.

According to Vanderplanten, damaged and exhausted powdered nutmegs are sometimes made up with some medium to cause the particles to adhere, into factitious nutmegs. These, however, are easily crushed to powder, especially when heated in water, and on cutting show no vegetable structure. No difficulty is experienced in detecting these when compared with a genuine nutmeg. An analysis of such spurious nutmegs gave the following figures:—

	Per cent
Moisture	11.09
Ash	11.34
„ insoluble in HCl	3.90
Ether extract	15.42
Essential oil	1.76
Cellulose	8.44

Genuine nutmegs will show the following characters on analysis, the estimation of mineral matter and fat being the principal useful chemical determinations, except when a large quantity is available, when the essential oil may be estimated:—

	Per cent
Moisture	4 to 8
Total ash	2 „ 4
Ash soluble in H ₂ O	0.8 „ 1.4
„ insoluble in HCl	0 „ 0.15
Fat	32 „ 36
Essential oil	5 „ 15
Alcohol extract	11 „ 17
Total nitrogen	1 „ 1.5
" Starch " (acid conversion)	15 „ 25

Any considerable reduction in the amount of fat indicates the presence of exhausted nutmegs.

Microscopic examination.—The uncoloured portion of powdered nutmeg under the microscope consists of minute angular cells, containing oil, and sometimes crystals of myristic acid; aleurone grains are to be found

and numerous small but distinct starch granules, which show a well-marked central depression round the hilum. In the more coloured portions of the powder much brown pigmentary matter is to be found but no starch and but little oil.

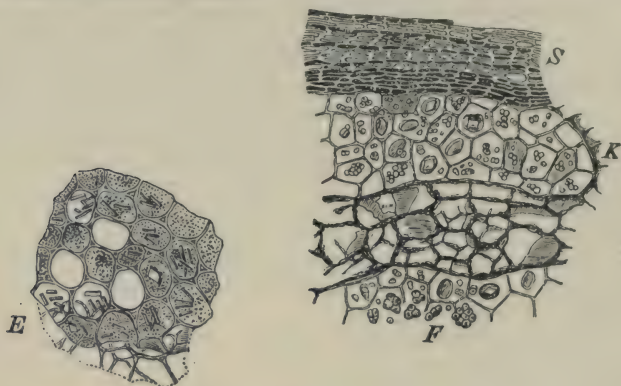


FIG. 32.—Powdered nutmeg.

The following illustrations represent the tissues present in nutmegs (after Möller).

K = endosperm cells with starch grains ; F = crystals of fatty acids ; E = cells with aleurone grains and crystals.

Nutmeg butter, which is a commercial article, is a mixture of about 5 per cent of the essential oil of nutmeg, with 95 per cent of fixed fat. Nearly all of the published figures for this fat are to be regarded with suspicion, as they have usually been obtained on the butter as found in commerce, and as this contains a variable amount of essential oil with a very high iodine value, any suggested methods of differentiating between the fat of Bombay and Banda mace or nutmegs depending on the iodine value, are useless.

The following figures are given by Spaeth :—

Origin.	M. Pt.	Sap. Value.	Iodine Value.	Refractometer No. at 40°.
Banda	25° to 26°	170 to 173	77·8 to 80·8	76 to 82
Bombay	31° „ 31·5°	189 „ 191	50·4 „ 53·5	48 „ 49
Menado	25·5°	169	76·9 „ 77·3	74 „ 74·5
Penang	26°	171·8 to 172	75·6 „ 76·1	84·5 „ 85
Macassar	25° to 25·5°	171·8 „ 172	75·6 „ 76·1	78·5
Zanzibar	25·5° „ 26°	169·9 „ 170·5	76·2 „ 77	77·5

An examination of these figures show that it is extremely probable that with the exception of the Bombay sample, they all contained notable proportions of essential oil. The author has examined three samples of pure commercial nutmeg butter, firstly in their natural state, and again after removing all traces of essential oil. The results are as follows :—

"Natural" Butter.				Freed from Essential Oil.		
	M. Pt.	Sap. Value.	Iodine Value.	M. Pt.	Sap. Value.	Iodine Value.
1	25°	174	76.5	29°	185	56
2	26°	172	74.9	29.5°	186	59.5
3	25°	175	78	30°	184	57.5

It does not appear that any great differences exist between the solid fats of the various species of mace and nutmeg.

Drs. Power and Salway, who have carefully examined the fixed oil of nutmeg, found that when the warmed Ceylon nutmegs were expressed, they yielded 26.6 per cent of fat, but that to ether they yielded 42.9 per cent. They found the nutmeg butter to contain a new unsaponifiable body of a viscid consistence, but without physiological action, to the extent of about 5 per cent, having the formula $C_{18}H_{32}O_3$. The other constituents of the expressed oil were trimyristin 73 per cent, essential oil 12.5 per cent, oleic acid as glyceride 3 per cent, linoleic acid as glyceride 0.5 per cent; formic, acetic, and cerotic acids, very small amounts, and unsaponifiable constituents 8.5 per cent; resinous material 2 per cent, and a little myristicin ("Journ. Chem. Soc." xciii. p. 1659).

The essential oil of nutmeg is official in the British Pharmacopœia, being used in the preparation of aromatic spirit of ammonia. It is there described as having a specific gravity of 0.870 to 0.910, and being soluble in an equal volume of 95 per cent alcohol. It should be free from the solid fat, as shown by leaving no crystalline residue when evaporated on a water bath. In the author's opinion these limits should be rather wider—from 0.868 to 0.915. The presence of traces of the fatty oil is said to be objectionable when the oil is used for the flavouring of *sal volatile* (aromatic spirits of ammonia). It may be detected by evaporating the oil, and purifying the residual crystals by washing them several times with cold alcohol and recrystallizing from boiling alcohol. The resulting *myristic acid* melts at 54° to 55°. They should be dextrorotatory from +14° to +40°, and should be soluble in an equal volume of 95 per cent alcohol. The chemistry of this oil requires elucidation.

The most reliable investigation of oil of nutmeg is that of Power and Salway ("Journ. Chem. Soc." 1907, 2037). They showed that it contained:—

	Per cent
Eugenol and iso-eugenol	0.2
Dextropinene and dextrocamphene	80
Dipentene	8
d-linalool }	about 6
d-borneol }	
i-terpineol }	
Safrol	0.6
Myristicin	4

and traces of alcohols, aldehydes, esters, and free acids.

MACE.

By mace is generally understood the arillus of the fruit *Myristica fragrans*, the nutmeg of commerce, which is official in the British Pharmacopœia. This is known as Banda mace and is usually regarded as the only genuine one. There are other maces, each being the arillus of another species of nutmeg, but the only ones which are seen in commerce are Bombay mace, derived from *M. Malabarica*, and Macassar or wild mace, derived from *M. argentea*. The so-called false mace, from *M. fatua*, is said to be sometimes met with, but this is improbable. Mace is usually sold in retail shops in the form of powder. Banda, or genuine mace, owes its characteristic fragrantcy and its value as a spice largely to an essential oil, which is absent or only present to a small extent in the other varieties of mace. It also contains a solid fat, which has the same characteristics as that obtained from the nutmeg and which is described on p. 234. A comparison of the figures there given shows that it is not possible to detect the presence of Bombay mace by the iodine value, but the low refractive value of the Bombay fat may give a useful indication. According to Leach, the following are the absolute refractive indices of the fats: Genuine mace, 1.4747 to 1.4975; Bombay mace, 1.4615 to 1.4633, at 35° C. Mace is sometimes adulterated with starchy matter, but the usual admixture is ground Bombay mace or wild mace. The following characters are the average of the results of a number of samples which were obtained as whole mace and ground in the laboratory:—

	True Mace.	Bombay Mace.	Macassar Mace.
	Per cent	Per cent	Per cent
Total ash . . .	1.87 to 2.36	1.9 to 2.1	1.7 to 2.08
Soluble in H ₂ O . .	1.08 „ 1.27	1.0 „ 1.2	1.1 „ 1.25
Insoluble in HCl . .	0.07 „ 0.2	0.07 „ 0.08	0.6 „ 0.075
Fixed ether extract .	25 „ 32.5	58 „ 63	49.5 „ 52
Alcoholic extract . .	22 „ 25	45.8	38
Volatile oil . . .	4 „ 8	4 to 8	4 to 7
Fibre	5 „ 9	3 „ 8	4 „ 8
Nitrogen	0.7 „ 1.2	0.8 „ 0.9	1 „ 1.2

The ether extract above given is that obtained by drying the mace, extracting with ether and drying at 110° until the volatile oil has been driven off.

In the examination of mace the following determinations should be made:—

Mineral Matter.—If the ash be substantially higher than 2 per cent the addition of mineral matter is probable. Such apocryphal adulterations as sawdust, which is stated to sometimes be found, would cause an increase in the ash. Of the ash at least 50 per cent should be soluble in water, and only a trace left insoluble in acid. If starch be added, the ash will be reduced.

Extracts.—Three quantitative extractions of the dried mace should

be made: (1) ether, (2) alcohol, (3) ether after exhaustion with petroleum ether.

In genuine mace, the ether extract, dried so as to drive off volatile matter, averages 22 per cent to 33 per cent, whereas in both Bombay and Macassar mace it is considerably higher—up to 63 per cent in the former and 52 per cent in the latter. The alcoholic extract, similarly dried, is seldom over 23 per cent in genuine mace, whereas it reaches 45 per cent in Bombay mace and 38 per cent in Macassar mace. A low alcoholic extract indicates the presence of exhausted mace. The extraction with ether, after exhaustion with petroleum ether, is very important. Genuine mace, extracted with petroleum ether, will only yield from 2 to 3·5 per cent of extractive to ether. Bombay mace, treated similarly, yields an extract up to 33 per cent, so that its presence in comparatively small amount is thus easily recognized. Macassar mace, on the other hand, behaves like Banda mace in this respect.

Umney gives the following figures for typical samples of known origin:—

	Petrol. Ether Extract.	Ether Extract after Petrol. Extract.
	Per cent	Per cent
Penang	17·55	2·68
Pale West Indian	22·71	2·04
Red „ „	28·37	3·90
Bombay	26·11	29·11

Griebel (*Zeit. Unterreich. Nahr. Genuss.* 1909, **18**, 202) gives the following method for the detection of Macassar mace in genuine mace: 0·1 gm. of the sample, and the same quantity of genuine mace are placed in test tubes and well shaken for one minute with 10 c.c. of petroleum spirit. The solutions are then filtered and 2 c.c. of each filtrate are mixed with 2 c.c. of glacial acetic acid in separate test tubes. Concentrated sulphuric acid is carefully added to both tubes so as to form a layer under the acetic acid solution. If the sample contains Macassar mace, a red ring forms at the junction of the two liquids, whilst with pure mace only a yellow colour develops. Two minutes should be allowed for the colour to develop. After this time even a pure mace may become red—hence the necessity of a check experiment on a pure sample. This test is useful, but should only be relied on as confirming more precise results obtained by quantitative determinations.

Special Reactions.—Schindler's reaction is useful in the detection of Bombay mace (*Chem. Central.* 1902, (2), 849): 5 grms. of powdered mace are packed, after being moistened with 8 c.c. of 98 per cent alcohol, into a percolating tube and placed over a suitable receiver; 8 c.c. more 98 per cent alcohol are added and the percolate collected. The receiver is changed and another similar quantity of alcohol added. This process is repeated several times. A drop of lead acetate solution is then added to the various receivers. With genuine mace, the first

tube shows a deep yellow-red precipitate; the second tube, less precipitate and of a paler colour; the third tube, none or only a slight whitish precipitate; and the fourth tube will be unaffected. With Bombay mace, a coloured precipitate results even from the 25th extraction. Hefelmann ("Pharm. Zeit." 1891, 122) recommends boiling the sample with alcohol and filtering through paper. The paper is stained red at the edge in the presence of Bombay mace; if only a small quantity is present, the stain may not appear until the paper is dried. If a slip of filter paper be moistened with an alcoholic extract of the mace, and a drop of weak caustic soda solution (decinormal) be added, a buff-pink colour results. In the presence of Bombay mace, this will be of a more or less deep orange.

If turmeric be suspected—it has occasionally been found—it can be detected by soaking filter paper in the alcoholic extract, drying and testing in the usual manner with boric acid.

Microscopical Examination.—Epidermal cells will be found to be very elongated and very thick-walled. The principal part will be found to consist of parenchymatous cells, mostly containing much fixed oil and a considerable amount of small granules of amylo-dextrin which stain red with iodine. Large receptacles or cells will be found which contain the essential oil. A few spiral vessels are present. No starch is present in pure mace.

If Bombay mace be present, the oil glands situated in the outer layers of this variety are very deeply coloured, so that deep red masses of resinous matter will be found, which are absent from ordinary mace.

Note.—Essential oil of mace is a pale or colourless oil of specific gravity 0.890 to 0.930 and having an optical rotation of about $+10^{\circ}$ to $+20^{\circ}$. It is soluble in 3 volumes of 90 per cent alcohol. It consists chiefly of terpenes, and some terpene alcohols, with a phenol and some myristicin (a complex benzene derivative).

COCHINEAL.

Cochineal is the dried impregnated female insect, *Coccus cacti*, which fixes itself firmly on to certain plants of the cactus family, especially the nopal, or *Nopalea coccinellifera*, an opuntia growing chiefly in Mexico. It is employed largely as a colouring matter both in foods and drugs.

The principal types of cochineal known in the market are the silver grain, the black grain, and granilla, the last named being, in all probability, the unimpregnated females.

The British Pharmacopœia, in which cochineal is official under the name *Coccus*, describes the insect as "about $\frac{1}{3}$ th of an inch long, somewhat oval in outline, flat or concave beneath, convex above, transversely wrinkled, purplish-black or purplish-grey, easily reduced to powder, which is dark red or puce-coloured. When cochineal is macerated in water no insoluble powder is separated. Incinerated with free access of air, it should yield not more than 6 per cent of ash."

Cochineal owes its colour to a complex acid of the probable formula $C_{11}H_{12}O_6$, named carminic acid, which is easily soluble in alkaline solutions. This, with certain other subsidiary bodies, is precipitated by such salts as alum or stannic chloride and then forms the carmine lake of commerce.

Under the name "liquid cochineal" is sold a fluid for colouring food preparations, and which is, in substance, an alkaline decoction of cochineal, preserved with more or less alcohol. The whole insect is frequently adulterated, either by the addition of exhausted insects or by dressing the natural insect with mineral matter rendered adherent in the wrinkles by means of a little gum. The British Pharmacopœia requires an ash value not exceeding 6 per cent, but Umney prefers 8 per cent as the maximum limit. It is probable that absolutely pure cochineal rarely has a higher ash value than 3 per cent. At all events plenty of cochineal is available with no higher ash value than that.

Cochineal—both partially exhausted and natural insects—is largely adulterated by facing with sulphate of barium, gypsum, mica, china-clay, and sometimes with bone-black and similar substances. All these adulterants raise the ash value considerably, and it is not uncommon to find samples with 12 per cent to 20 per cent of ash. The "silver grain" variety is usually faced with some finely powdered siliceous matter.

Exhausted cochineal always appears deeply wrinkled and is generally slow to absorb water—so that on throwing the exhausted insects into water, many of them will float for a time.

Apart from a determination of the ash value, the valuation of the insect from a colour point of view is the only method of forming an opinion on the quality of cochineal. This may be done by boiling 1 gm. of the powdered sample with 1 litre of water and 0.5 gm. of alum for an hour. On cooling, the solution is made up to 1 litre and the colour compared in Nessler glasses with standard specimens.

Merson ("Chemist and Druggist," 56, 517) recommends valuing cochineal from the colour point of view, by noting the amount of solution of chlorinated soda (containing 1 per cent of available chlorine) necessary to decolorize the colouring matter of 1 gm. of the sample. He finds that the best samples require over 20 c.c. whereas the poorest samples only require 9 to 10 c.c. He proposes the following method of carrying out the determination:—

Weigh 0.5 gm. of finely powdered cochineal; place in a 100 c.c. flask with 30 c.c. of distilled water, and 5 drops of strong ammonia; heat to boiling-point, strain through cotton-wool into a 100 c.c. flask, and wash with sufficient water to produce 100 c.c. The marc on the wool should now be quite colourless. Put 25 c.c. of the liquid into a 100 c.c. stoppered test-mixer, add 5 c.c. of strong hydrochloric acid, and sufficient distilled water to produce 100 c.c. Run in 0.5 c.c. at a time of solution of chlorinated lime (or soda), containing 1 per cent of available chlorine, till the cherry-red colour changes to dull orange, shaking briskly after each addition. Continue adding chlorinated solution in 0.1 c.c. portions as long as the colour is being bleached.

When almost completed, note the burette reading, and after adding a further 0.1 c.c. of solution, shake the liquid slightly and see if the top layer is lighter than the lower. If there is no difference, the reaction is finished; if the lower stratum is darker, continue to add chlorinated solution drop by drop till the action is quite complete.

An approximate determination of the amount of colouring matter may be made by exhausting the cochineal with boiling water, precipitating the colouring matter with a slightly acid solution of acetate of lead, and washing and drying the lead precipitate. The lead precipitate is ignited in a porcelain dish and the loss on weight returned as colouring matter.

Lagorce recommends the following method for detecting cochineal in alimentary substances.

The substance should be dissolved in water or weak alcohol faintly acidified with acetic acid. The liquid is shaken with amyl alcohol, which is separated and evaporated in the presence of water. The water solution so obtained is treated with a few drops of a 3 per cent solution of uranium acetate, when a beautiful bluish-green colour or precipitate will be produced if cochineal be present. Acids destroy this colour, with production of the orange tint of the carminic acid. In the case of wine, a mixture of amyl alcohol and toluene should be used.

Logwood is distinguished by the black colour produced with ferrous sulphate, and brazil wood by adding excess of lime water to a little of the solution. This completely precipitates the colouring matter of cochineal, but if brazil wood be present, the filtered liquid will have a purple or violet colour.

SAFFRON.

Saffron consists of the dried stigmata, together with the tops of the styles, of *Crocus sativus*. It is used, especially in certain parts of the country, as a colouring matter for cakes, and is also employed in medicine. Under the name "*Crocus*" it is official in the British Pharmacopœia.

That authority requires it to have the following characters: water, not to exceed 12.5 per cent; ash, not more than 7 per cent. On incineration it does not deflagrate (absence of nitrates). When pressed between blotting paper it does not leave an oily stain. When a small portion is placed in a glass of warm water it colours the water orange yellow, becomes paler itself in colour and does not deposit any white or coloured powder.

Saffron contains about 0.6 per cent of an aromatic essential oil, and a colouring matter, known as crocin, of the empirical formula $C_{44}H_{70}H_{28}$. It probably contains a second colouring matter, known as picrocrocin $C_{28}H_{66}O_{17}$.

Saffron is adulterated to a very large extent, the greater part of that known as Alicante saffron being a mixture of genuine saffron and other fibres dyed with a coal-tar colour. Maisch ("Analyst," x. 200) has given an excellent account of the adulterations of saffron. These

consist, as a rule, of either dyed or naturally coloured fibres, or of mineral matter.

Moisture and Ash.—The moisture in commercial saffron averages from 9 to 12 per cent or should not exceed 12·5 per cent. Occasionally a trace of glycerine is added in order to induce the saffron to absorb moisture from the atmosphere. The mineral matter varies from 4·5 to 7 per cent—rarely reaching 8 per cent, of which not more than 0·5 per cent is siliceous. No deflagration should take place during incineration, or nitrates are indicated, and should be tested for in an aqueous extract. Nitrate of potash is often added, in a strong aqueous solution, to increase the weight of the saffron. In addition to this, chalk, sulphates of lime or barium, sulphate of soda, and other salts are sometimes found, being rendered adherent with a trace of glycerine or glucose (a conviction was obtained in London in 1909 for the adulteration of saffron with barium sulphate).

If the sample is suspected of being weighted with a mineral, it is recommended to be placed on the surface of water and gently stirred, when the water immediately becomes turbid and gradually the powder subsides, if allowed to stand. In all samples a small quantity of pollen thus deposits, but its nature can be detected under the microscope. The nature of any soluble mineral matter present may be ascertained by testing the aqueous infusion for ammonium salts, nitrates, etc., in the usual way. The insoluble salts in the deposit have to be rendered soluble by fusion with alkaline carbonates and then examined according to the ordinary rules of mineral analysis.

Other Fibres.—The principal fibres which have been recorded as adulterants of saffron are as follows: the corolla tubes and stamens of the *Crocus*, dyed with either brazil or santal wood dye; or with a coal-tar yellow (usually dinitrocresylate of sodium); *Calendula*, *Carthamus*, *Cyanara*, red poppy, threads of algæ, and various other plant fibres.

For the general detection of such adulterations, the sample should be scattered on the surface of warm water. The genuine saffron fibres at once expand to a characteristic form, which are readily distinguished from *Crocus* stamens, and such fibres as *Carthamus* florets, or *Calendula* florets. A comparison with a standard sample will enable most adulterations to be thus readily detected.

Kraemer recommends adding the sample to dilute sulphuric acid.

With *crocus* only the stigmas become blue immediately, and in half a minute the solution becomes blue, gradually changing first to a violet, then to a deep wine-red colour. The flowers of *Carthamus* turn yellow; the solution remains colourless for a few minutes, then becomes yellow, and after a much longer time assumes a deep wine-red colour. *Calendula* flowers turn brown, or blackish-brown, as if charred, but the solution behaves much the same as with *Carthamus*.

The colouring matter from santal wood is characterized by being soluble in alcohol with a red colour, and in ammonia with a purple-red colour. Brazil and logwood dyes will tinge the water a red colour, deepening by addition of ammonia and becoming paler by addition of acid. Coal-tar dyes may be usually detected as follows:—

Nitrocresylate of sodium, which is the dye most usually employed, may be detected by soaking in petroleum spirit, when the spirit acquires a lemon-yellow colour, the colouring matter of saffron not being soluble in that liquid.

According to Wanters ("Bull. Assoc. Belge. Chem." xii. 103) a good test consists in trying the tinctorial power of a sample on wool, silk, and cotton. These materials strike a citron-yellow colour with a solution of the true drug, containing tartaric acid, which is not altered by subsequently treating the materials with potassium hydrate. Under treatment with a solution of the spurious article, the wool takes a deep brownish-red, the silk a deep orange-yellow, and the cotton a lighter yellow tint; in each case the addition of potassium hydrate causes a deepening of colour.

Pfyl and Scheitz ("Zeit. Nahr. Genuss." 1908, 16, 347) estimates the value of saffron by determining the amount of sugar obtained by hydrolysing the chloroform-soluble glucosides present. The sample is dried, and 5 grms. of the dry, powdered saffron are extracted in a Soxhlet with petroleum ether, and after drying with chloroform, the solvent is evaporated from the chloroformic solution and the residue taken up as far as possible in acetone. The latter is evaporated and the glucoside in the residue hydrolysed by adding 5 c.c. of normal HCl, and heating for fifteen minutes, water being added as required, to bring the amount of the liquid up to 25 c.c. The liquid is, if necessary, filtered, and neutralized with normal alkali, and the sugar estimated by reduction of Fehling's solution, the copper being weighed as CuO, and calculated to Cu.

Pure saffron, consisting only of stigmata of the crocus, yields from 0.199 grm. to 0.209 grm. of Cu, when treated in this manner.

The styles of the Crocus, logwood, poppy petals, peony petals, marigold, safflower, Cape saffron, and Spanish thistle flowers give practically no copper, and turmeric and red sanders wood very much less than saffron.

Microscopic Examination.—The principal tissues consist of long-celled parenchyma, with a number of vessels, often spiral; and some of the parenchymatous cells have large papillæ attached, and nearly all the cells are red in colour. A comparison with genuine saffron will enable most adulterants to be detected under the microscope.

Valuation of Saffron.—Proctor has suggested a colorimetric method of valuing saffron from a colour point of view. Dowzard has also worked in this direction and gives the following method ("Ph. Jour." 4, vii. 443) which, in the author's opinion, is fairly accurate:—

The method is not designed to furnish evidence of adulteration, but merely to test the value of a sample of saffron as a colouring agent.

A standard solution of chromic acid is prepared containing 78.7 grms. of chromic acid per litre. One hundred c.c. of the above solution are equal in tinctorial power to 0.15 grm. of crude crocin dissolved in 100 c.c. of water (crude crocin is obtained by extracting saffron with ether, drying, and exhausting the residue with 50 per cent alcohol; the alcoholic solution is evaporated to dryness, and the residue taken as crude crocin).

The sample is reduced to a coarse powder by pestle and mortar, 0.2 gram. of the powder is transferred to a stoppered cylinder having a capacity of about 35 c.c.; 20 c.c. of 50 per cent alcohol are then introduced into the cylinder, which is tightly stoppered, and placed in water at 50° C. for $2\frac{1}{2}$ hours. The solution is cooled and filtered, 10 c.c. of the filtrate (= 0.1 gram. of saffron) are diluted with water to 50 c.c., and the depth of colour compared with 50 c.c. of the standard chromic acid solution (for comparing the colours it is sufficient to use two Nessler glasses of equal bore). If the chromic solution is deeper in tint than the solution under comparison, small quantities are removed until equality is produced, or vice versa; the solutions are then measured, and the amount of crude crocin calculated.

Example.—Ten c.c. of saffron solution (= 0.1 gram. of saffron) diluted to 50 c.c. had a depth of colour equal to 40 c.c. of the standard chromic acid solution.

$$100 : 40 :: 0.15 : x$$

$$= 0.06.$$

$$\therefore 50 \text{ c.c. contain } 0.06 \text{ gram. of crude crocin.}$$

$$0.1 : 100 :: 0.06 : x$$

$$= 60.0.$$

The above example therefore contains 60 per cent of crude crocin.

The finest samples on the market contain upwards of 75 per cent of crude crocin; good samples of saffron should not contain less than 50 per cent.

TURMERIC.

Turmeric is the rhizome of *Curcuma longa*, or so-called Indian saffron (the name is derived from the word Kurkum, the Persian word for saffron), and *Curcuma rotunda*. Powdered turmeric is used to a very large extent in the preparation of curries, pickles, etc., the characteristic colour and flavour of "picallili" being produced by turmeric. It is usually sold in shops in the powdered form. Turmeric contains about 10 per cent of a resin, and about 5 per cent of an essential oil having the characteristic odour of the rhizome. It owes its colour to a substance named curcumin, probably bearing the formula $C_{19}H_{14}O_4$ (OCH_3)₂. For the chemistry of this body see Ciamician and Silber ("Berichte," xxx. 192).

In the table on p. 244 A. E. Leach ("Journ. Amer. Chem." Soc. 26, 1210), gives the analyses of the three varieties of turmeric most frequently met with in commerce.

To curcumin the characteristic boric acid reaction is due. The reaction is best applied to blotting-paper soaked in an alcoholic extract of the turmeric and dried. A solution containing boric acid, or borax to which sufficient HCl has been added to show an acid reaction to litmus, is spotted on to the paper and the latter again dried. The spot will be of a red colour, changed by a drop of alcohol through a very varying series of colours, in which green and purple predominate.

According to Bell ("Pharm. Journ." 4, 15, 551) turmeric may be

Variety.	Moisture.	Total Ash.	Ash Soluble in Water.	Ash Insoluble in HCl.	Total Nitrogen.	Protein; N \times 6.25.	Total Ether Extract.	Volatile Ether Extract.	Non-volatile Ether Extract.	Alcohol Extract.	Crude Fibre.	Reducing Matters by Acid Conversion of Starch.	Starch by Diastase Method.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
China	9.03	6.72	5.20	0.11	1.73	10.81	10.86	2.01	8.84	9.22	4.45	48.69	40.05
Pubna	9.08	8.52	6.14	—	0.97	6.06	12.01	4.42	7.60	7.28	5.84	50.08	29.56
Aleppo	8.07	5.99	4.74	—	1.56	9.75	10.66	3.16	7.51	4.37	5.83	50.44	33.03
Average	8.73	7.07	5.36	—	1.42	8.88	11.17	3.19	7.98	6.96	5.37	49.73	34.21

identified in mixtures by its reaction with diphenylamine. It gives with diphenylamine, in acid alcoholic solution, a fine purple coloration. No other vegetable colouring matter has been found to give a similar reaction, so that the test is available for turmeric mixed with other substances. One part of turmeric in 200 parts of rhubarb, or in 1000 parts of mustard, is readily detected.

The following is the best method of applying the test: A drop of the reagent is placed on a clean microscopic slide by means of a glass rod, a small quantity of the powder under examination is spread evenly over the entire surface of a cover glass, and carefully dropped over the reagent on the slide.

The slide is then examined microscopically with an inch objective, when, if turmeric be present, spots of a fine purple colour will be observed scattered throughout the field. The number of these purple spots can be employed in estimating approximately the amount of the drug present by comparison with standard specimen slides containing a known percentage of turmeric.

The reagent consists of pure diphenylamine, alcohol 90 per cent, and pure sulphuric acid:—

Diphenylamine	1 gm.
Alcohol 90 per cent	20 c.c.
Pure sulphuric acid	25 c.c.

The diphenylamine is dissolved in the 90 per cent alcohol, and the sulphuric acid is then added. When cold the reagent is ready for use.

In examining turmeric, the following determinations will afford most of the necessary information:—

Moisture.—The average amount of moisture is 8 per cent to 9 per cent. Excess will cause the sample to cake and it will then rapidly deteriorate.

Ash.—The total ash of turmeric varies from 6 per cent to 8.5 per cent of which 70 per cent is soluble in water, and not more than 0.2 per cent insoluble in hydrochloric acid.

Extractives.—The ether extract, free from essential oil, varies from 7·5 per cent to 10 per cent, the alcoholic extract from 5·5 per cent to 10 per cent.

Starchy Matter.—Turmeric contains a considerable amount of starch, and if this is converted in the usual manner by acid, the amount of glucose obtained by reduction of Fehling's solution should vary from 47 per cent to 52 per cent. Excess of this indicates adulteration with starchy matter.

Microscopic Examination.—The mass of the tissue consists of large yellow parenchymatous cells filled with colouring matter; many starch granules are present, some stained yellow by curcumin, and swollen

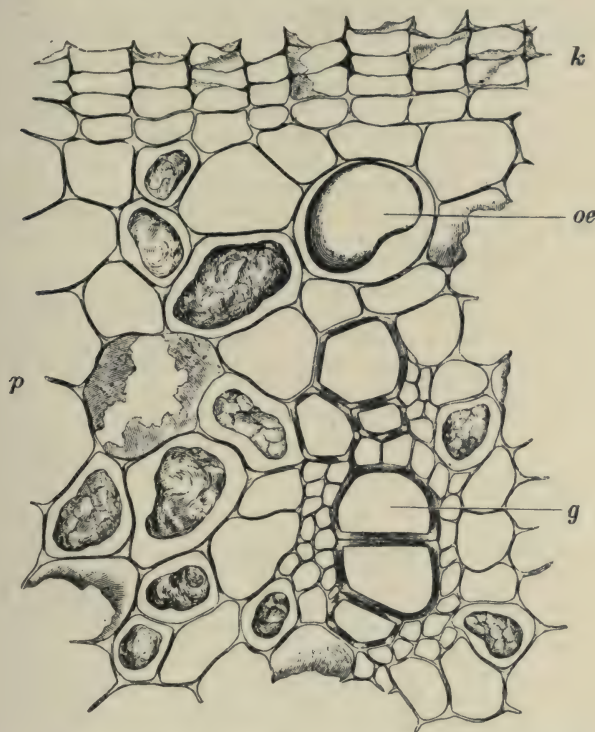


FIG. 33.—Turmeric rhizome, transverse section, *g*, vessel; *k*, cork; *oe*, oleo-resin; *p*, parenchyma of wood, containing masses of gelatinized starch. (Möller.)

by the process of scalding to which the rhizome is usually subjected before being put on the market. The starch granules are oval, oblong or oyster-shaped and show well-defined rings and an eccentric hilum, but, owing to the method of preparation, the starch grains are usually more or less disintegrated, and well-defined starch grains may indicate the presence of an adulterant. The microscopic appearance is very

similar to that of ginger, but there are no bast fibres. Foreign starches should be searched for.

Oil of Turmeric.—This is a thick liquid of specific gravity about 0·940 and contains an alcohol termed turmerol, which is probably the aromatic ingredient of the oil. It is of little commercial importance.

ANNATTO.

Annatto is a colouring matter employed in the preparation of various foods, to which a pale yellow tint is imparted. It consists essentially of the soft tissue surrounding the fruits of *Bixa orellana*, a plant growing in the East and West Indies and in South America. Such American annatto usually arrives in this country in the form of hard rolls or cakes, containing from 15 to 30 per cent moisture, whereas Cayenne annatto is generally imported in the form of a soft paste. It owes its colouring power to at least two bodies, bixin $C_{28}H_{34}O_5$, and orellin, of unknown composition. Annatto consists of about 25 per cent of colouring matter, with cellular tissue, glutinous matter, and mineral matter.

It is soluble to a variable extent in water, and to a greater extent in alcohol. Caustic alkalis, alkaline carbonates and borax in solution dissolve it to a large extent, orange-red colouring matter being precipitated on the addition of acids.

Annatto is adulterated to a considerable extent, the principal adulterants being starchy matter, oxide of iron, salt and aniline dyes. The last named are added, together with a little potassium carbonate, to improve the colour of samples which have been reduced with starchy matter or similar adulterants.

Pure roll annatto has the following composition:—

	Per cent
Water	15 to 20
Resinous matter	23 „ 30
Mineral matter	18 „ 22
Extractive matter	20 „ 28

Paste annatto has a proportional composition, with water up to 70 or 75 per cent.

Lawson ("Pharm. Journ." [3], xvi. 645) gives the following as his results of the analysis of a number of commercial samples:—

Moisture.	Resin.	Extractive.	Total Ash.	Ash Soluble in H_2O .	
Per cent	Per cent	Per cent	Per cent	Per cent	
21·75	3·00	57·29	17·96	13·20	roll
21·60	2·90	59·33	16·17	12·57	„
20·39	1·00	65·00	13·61	7·50	„
69·73	8·80	19·47	2·00	—	paste roll
18·00	3·00	58·40	20·60	10·00	
18·28	1·80	65·67	14·25	11·75	
15·71	5·40	26·89	52·00	18·50	
38·18	1·20	20·82	29·00	20·00	
19·33	5·99	23·77	51·00	15·00	
29·50	9·20	28·50	39·80	13·80	

Analysis of Annatto.—The water should first be determined, and the dry residue should be exhausted with boiling methylated spirit. After driving off the alcohol, the residue is re-dissolved in a solution of sodium carbonate and a slight excess of dilute sulphuric acid added to the liquid. The resin is precipitated, and is filtered off, washed with water, dried, and weighed.

The ash is determined on a fresh portion of the sample.

The extractive matter (which however includes a little insoluble matter) is taken as the difference.

Annatto should be examined microscopically. Owing to its method of treatment, but little structure will be observed, only a few starch granules being unbroken. The presence of added starchy matter or turmeric is therefore fairly easily identified.

In solution of colouring matter in which annatto is suspected, the following reaction may be applied :—

A dilute solution of the colouring matter is floated in a test tube, on an equal volume of dilute HNO_3 , so that the two solutions do not mix. In the presence of annatto the zone of contact at once shows a deep blue colour, the colour spreads into the HNO_3 , which soon becomes green, and the upper aqueous layer shows a reddish turbidity.

Milk is often coloured with a trace of annatto to give it a fictitious appearance of richness. To detect this addition, Leys employs the following test: 50 c.c. of the sample are shaken out with twice its volume of ether-alcohol mixture composed of 240 parts of alcohol (93 per cent), 320 parts of ether, 20 parts of water, and 8 parts of solution of ammonia of .920 specific gravity. After separation, the ethereal layer is rejected, the colouring matter being retained in the aqueous portion. This is transferred to another vessel and half its volume of a 10 per cent solution of sodium sulphate gradually added, which causes slow separation of the casein. The clear aqueous portion is decanted and shaken out with amylic alcohol, the washing being conducted in test-tubes to facilitate the separation of the solvent. After shaking, these tubes are plunged into a cold water bath, the temperature of which is gradually raised to 80°C. , when separation will be complete. The amylic alcohol solution is collected and evaporated. The deep yellow residue is re-dissolved in warm water containing a little ammonia and alcohol, a strip of bleached cotton is immersed in the solution, and the whole evaporated to dryness. The cotton, which is now of a yellow tint, is washed and plunged into a solution of citric acid. If the colouring be annatto, the thread will at once assume a marked rose tint. Uncoloured normal milk imparts a very slight yellow tint to cotton by this method, but does not give the change of tint with citric acid, which is characteristic of annatto.

VINEGAR.

The definition of vinegar is by no means an easy matter. Originally, as is obvious from its name ("sour wine"), vinegar was the product of the acetous fermentation of wine. To-day products which are essentially of the same character are obtained by similar fermenta-

tions of other alcoholic liquids, and to these the term vinegar is naturally applied. The characteristic constituent of vinegar is acetic acid, and the name has therefore been extended—rightly or wrongly—to dilute acetic acid obtained by the distillation of wood. The propriety of thus extending the name vinegar will be discussed in the second volume of this work, dealing with the law on the subject. In the present chapter, wood vinegar will be understood to be excluded from the term vinegar, except when qualified by the word “wood” as denoting its origin.

With this qualification vinegar may be defined as the product, consisting of dilute acetic acid with small quantities of subsidiary constituents, obtained by the oxidation of alcoholic solutions obtained from vegetable sources.

In practice this oxidation takes place by means of fermentative changes brought about by an organism known as *mycoderma aceti*. The principal varieties of vinegar are as follows:—

(1) *Malt Vinegar*.—This is the product of the fermentation of a wort made from malt and barley (but see below).

(2) *Wine Vinegar*.—This vinegar is made from grape juice and low-grade red or white wines.

(3) *Sugar Vinegar*.—This is usually made by the hydrolysis of starchy matter by means of dilute acids, followed by fermentation of the starch, and subsequent acetification of the alcohol formed.

(4) *Cider Vinegar*, made from cider (or perry).

(5) *Date vinegar*, made from a fermented extract of dates.

The general characters of vinegars made from various sources differ somewhat and are shown in the table on opposite page.

Fairley (“Analyst,” xxxiv. 515) has shown that malt vinegars, brewed from mixtures of malted and unmalted grain (maize in this instance) will often contain less phosphoric acid than some whole malt vinegars. He gives the following figures for a number of samples:—

	Average.	Maximum.	Minimum.
	Per cent	Per cent	Per cent
Acetic acid . . .	4.50	5.30	3.65
Total solids . . .	2.51	4.01	1.52
Ash	0.45	0.96	0.14
Proteins	0.40	0.79	0.22
P ₂ O ₅	0.058	0.083	0.040
Specific gravity . .	1.0169	1.0210	1.0120

How far such vinegars are properly described as “malt vinegar” is a matter which has never been decided in a Law Court. The following remarks, due to Dr. Bernard Dyer, probably express the current opinion on the subject (“Analyst,” xxxiv. 518):—

“He imagined that there were two accepted meanings for the term “malt vinegar”. The view of the extreme purist would be that malt vinegar was vinegar in which everything but the water was de-

rived from malt. The more liberal interpretation, and one which for a number of years had been largely accepted, was that malt vinegar

	Malt.	Wine.	Sugar.	Cider.	Date.	Rice.
	Per cent 1-014 to 1-023	Per cent 1-013 to 1-023	Per cent 1-009 to 1-012	Per cent 1-012 to 1-016	Per cent 1-017 to 1-021	Per cent 1-016 to 1-020
Specific gravity	1-5 " 3-5	1-4 " 3-32	1-4 " 1-7	1-9 " 2-2	2-2 " 3-1	2-4 " 2-9
Solid matter	0-18 " 0-60	0-16 " 0-6	0-2 " 0-3	0-2 " 0-4	0-4 " 0-5	0-2 " 0-35
Mineral matter	3-8 " 6-5	6 " 10	4 " 5	2-5 " 6	5-8 " 6-6	5 " 6
Acetic acid	0-088 " 0-125	0-06 " 0-1	0 " 0-01	0-04 " 0-07	0-06	0-01 " 0-02
Nitrogen	0-04 " 0-14	0-01 " 0-02	traces	0-01 " 0-035	0-04	0-015 " 0-02
Phosphates as P_2O_5	0-016 " 0-085	—	traces	0-15 " 0-18	—	0-01 " 0-02
Alkalinity of ash as K_2O						

was vinegar in which the acid was derived from starch which had been rendered soluble by the diastatic action of malt—i.e. vinegar made by means of malt, but not necessarily wholly from malt. Malt

vinegar, even according to this liberal interpretation, would differ from vinegar made from sugar or from a mixture of malt and sugar."

In addition to acetic acid, vinegar contains such bodies as ethyl acetate, traces of alcohol, sugar, gummy matter, acetates, tartrates and phosphates, colouring matter, albumenoids and traces of some bodies which impart the characteristic odours to the different vinegars.

Vinegar is known to English manufacturers by numbers—18, 20, 22 and 24 being the usual strengths. These numbers refer to the amount of dry sodium carbonate (in grains) required to neutralize one ounce of the vinegar. Since 60 grains of acetic acid are neutralized by 53 of sodium carbonate, the number of grains of acetic acid per ounce corresponds with the number of the vinegar multiplied by 1.132. The "number" multiplied by 0.259 gives the percentage of acetic acid (i.e. *weight* of acetic acid in 100 *volumes* of the vinegar).

Before discussing the analysis of vinegar, it must be recognized that an expert nose and palate will often be of more value than a chemical analysis, since factitious vinegars made from dilute acetic acid, coloured with caramel, with a trace of phosphates and nitrogenous matter are made which give results practically indistinguishable from those of genuine vinegars.

The analysis of vinegar should include the following determinations:—

Acetic Acid.—Acetic acid may be determined with sufficient accuracy by titrating a measured quantity with standard potash solution, using phenol-phthalein as an indicator. The sample should be diluted—say 5 c.c. to 50 c.c.—with distilled water and titrated with semi-normal alkali.

Any refinements, such as adding excess of sodium carbonate and titrating back with standard acid with the use of cochineal as indicator are quite unnecessary.

The only error likely to occur is due to the presence of mineral acids in the sample. Very weak vinegar is liable to putrefy, and on this account an old excise regulation (under the Vinegar Act of 1818 since repealed) allows the addition of 1 gallon of sulphuric acid per 1000 gallons of vinegar. This addition is not necessary in good vinegars and is not often practised, although it is not yet an obsolete custom. The deliberate adulteration of vinegar with sulphuric or hydrochloric acid is not a very common occurrence.

Free hydrochloric acid may be determined by distilling 100 c.c. of the sample till 90 c.c. have been collected, adding 100 c.c. of distilled water to the residue in the distillation flask and distilling a further 100 c.c. Practically the whole of the hydrochloric acid will be found in the distillate and may be precipitated and weighed as silver chloride.

Hehner has examined this subject and has suggested a method for determining the free mineral acids. Vinegar always contains potassium and sodium salts of acetic acid (and tartaric acid, if present). Mineral acids added in *small* quantity will therefore merely decompose such salts and become converted into sulphates or chlorides as the case may be, disappearing as free mineral acids entirely. Any excess, however,

over the quantity necessary for such reaction remains as free mineral acid.

It is therefore clear that if any undecomposed acetate or tartrate remains in the vinegar, it is impossible for any free mineral acid to be present. To decide this point, the vinegar should be evaporated and the ash tested. Acetates and tartrates yield carbonates on ignition, and therefore if the ash is alkaline, no free mineral acid could have been present. If the ash is neutral, free mineral acid is *probably* present. To use this as a quantitative reaction, a known volume of the vinegar should be carefully neutralized with standard solution of soda, and the sample now evaporated to dryness. The residue is ignited and the aqueous solution of the ash is titrated with standard acid. If the original acid of the vinegar were entirely organic, the ash will require for neutralization acid exactly equivalent to the alkali required for the neutralization of the vinegar. Any deficiency in the amount of the standard acid required for neutralization of the ash is due to free mineral acid originally in the vinegar. Fifty c.c. may be used for this determination. Free sulphuric acid may also be determined by evaporating 100 c.c. of the vinegar to a very small bulk and adding to the liquid when cold four or five times its volume of alcohol. Sulphates are precipitated, free sulphuric acid remaining in solution. The filtered liquid is diluted with water, the alcohol driven off, and the free sulphuric acid precipitated as barium sulphate. If much chloride was present in the water used for manufacturing the vinegar, some of the added sulphuric acid will have decomposed the chlorides, and free hydrochloric acid will also be present, which must be estimated by distillation.

In regard to qualitative reactions, it must be remembered that many waters used for brewing vinegar contain large amounts of chlorides and sulphates, so that reactions for these salts are meaningless.

To detect free mineral acids Ashby ("Analyst," ix. 96) prepares a 2 per cent decoction of logwood. Drops of this liquid are spotted on a flat porcelain plate, and evaporated to dryness over boiling water. A drop of the suspected sample (concentrated if necessary) is added to a spot of the logwood decoction and evaporated. In the presence of free mineral acids, the residue is bright red, whereas in the presence of pure vinegar it will be bright yellow.

Hilger ("Archiv. des Pharmazie," 1876, 193) adds a few drops of a 0.1 per cent solution of methyl-violet to 25 c.c. of the vinegar. If this be pure no change in colour results, but in the presence of as little as 0.2 per cent of free mineral acid, the colour becomes blue; with 0.5 per cent it is blue-green, and with 1 per cent it is green.

Total Solid Matter.—Ten c.c. of vinegar should be evaporated to dryness on a water bath, and as the residue obstinately retains traces of acetic acid, it is best to add a little alcohol to this and evaporate again to constant weight. The extract varies according to the nature of the vinegar, as will be seen from the table on p. 249.

Mineral Matter.—The residue obtained in determining the total solid matter is ignited at as low a temperature as possible, and the ash

weighed. The average amounts for the various types of vinegar will be found in the table on p. 249. Sugar vinegar, prepared by the inversion of starches, yields an ash containing much sulphate. Cane-sugar vinegar yields a readily fusible ash, whilst that of a malt or glucose vinegar is not readily fusible. The ash of cane-sugar vinegar is composed chiefly of potassium salts.

Nitrogen.—Vinegar made from sugars contains hardly any proteid matter. Grain vinegars, on the other hand, contain as much as 0.75 per cent of proteids. To determine the nitrogen 25 c.c. may be evaporated to a syrupy consistency and the nitrogen determined by any modification of the Kjeldahl method. A genuine malt vinegar will contain from 0.088 to 0.125 per cent of nitrogen, whereas sugar vinegars often contain less than 0.01 per cent, and factitious vinegars made from acetic acid practically none at all.

Phosphoric Acid.—The phosphoric acid should be determined by treating the ash with a little hydrochloric acid and evaporating to dryness. The residue is then dissolved in about 10 drops of dilute acetic acid; 50 c.c. of boiling water are added, and about 1 grm. of sodium acetate. This solution is then titrated with uranium acetate solution in the usual manner.

Metallic Contamination.—Occasionally traces of copper, lead, zinc, or tin are found in vinegar. To detect these, 10 c.c. of the sample is boiled and 1 c.c. of strong hydrochloric acid is added. A little potassium chlorate is added in very small quantities at a time, whilst the liquid is still boiling, until the colour becomes pale yellow: the boiling is continued for a minute, and the liquid is then treated with sodium acetate, to remove the hydrochloric acid, and treated with H_2S . Very faint traces of lead, copper, or tin can be thus detected.

Colouring Matter.—Factitious vinegar is usually coloured with caramel. Amthor ("Zeit. f. Anal. Chemie," xxiv. 30) recommends the following process for its detection: 10 c.c. of the sample and 30 c.c. of paraldehyde are mixed with sufficient absolute alcohol to obtain complete solution. The mixture is allowed to stand in a closed vessel for twenty-four hours, when caramel, if present, will be precipitated. The liquid is decanted, the precipitate washed with a little absolute alcohol, dissolved in water, and the aqueous solution evaporated to 1 c.c. To this a small quantity of a solution of phenylhydrazine in acetic acid is added and the liquid heated to 100° for half an hour. An amorphous compound is formed, which is probably a mixture of phenylhydrazones and osazones, if the precipitate were caramel.

No accurate method is known for the estimation of caramel, nor, as a rule, is one required. It has been suggested that colorimetric tests, using caramel as a standard, give fairly accurate results, but this is not so, as the colour of caramel varies so much, according to its method of preparation, that one sample may be of a much greater depth of colour than another—so that quantitative comparisons are impossible.

Preservatives.—Preservatives are seldom added to vinegar (see above as to the addition of sulphuric acid). Salicylic acid is, how-

ever, occasionally found to be present. It may be detected by adding a few drops of sulphuric acid to the sample, and extracting with ether or petroleum ether, and estimated colorimetrically by means of iron-ammonia alum (see page 680).

Benzoic acid can be detected by extracting in the same manner, and, after washing the ethereal extract with water, evaporating the ether on a watch-glass and covering the extract with a piece of filter paper and then with a clock-glass. If the watch-glass be heated over a small flame, benzoic acid will sublime through the paper and collects on the upper glass. The crystals are characteristic in form and may also be recognized by their reaction towards ferric chloride.

The Detection of Methyl-acetol.—Pastureau has observed that specimens of commercial vinegar give precipitates with 95 per cent alcohol and possess a powerful reducing action *in the cold* on alkaline cupric tartrate. He finds this to be due to the presence of methyl-acetol, $\text{CH}_3\text{CO}\cdot\text{CHOH}\cdot\text{CH}_3$, in one case to the extent of .325 per cent. This substance should not be present in naturally brewed vinegars, and may possibly indicate the presence of wood distillation products. To determine this substance 50 c.c. of the vinegar are neutralized with Na_2CO_3 and distilled carefully to dryness. The distillate is collected in a graduated 100 c.c. flask and is rendered alkaline by a few drops of caustic soda solution and ammonia and then treated with 10 c.c. of decinormal solution of silver nitrate. After being allowed to stand for twenty-four hours, the liquid is made up to 100 c.c. with water, filtered, and the excess of silver determined by the potassium cyanide method. The amount of methyl-acetol present is calculated from the amount of silver reduced, according to the equation:—

$$3(\text{CH}_3\text{CO}\cdot\text{CHOH}\cdot\text{CH}_3) + \text{AgNO}_3 = 3(\text{CH}_3\text{CO}\cdot\text{CO}\cdot\text{CH}_3) + 3\text{H}_2\text{O} + \text{N} + \text{Ag}.$$

REMARKS ON SPECIAL TYPES OF VINEGAR.

Wine Vinegar.—This vinegar varies in colour, according to the type of wine from which it has been made. Further, the darker varieties are often distilled, and the product sold as “distilled wine vinegar”. Naturally this type of vinegar is practically free from solid matter, and can only be distinguished from a factitious vinegar by its fine characteristic aroma. Distilled wine vinegar is often described as white wine vinegar, but according to some, the latter term should be restricted to normal vinegar *made from white wine*. This is usually of a pale straw colour.

Normal wine vinegar contains from 5 to 10 per cent of acetic acid—usually from 6 to 8 per cent. Its specific gravity is usually fairly low, averaging about 1.017. If the solid residue (averaging about 2 per cent) be treated with absolute alcohol, nearly the whole is dissolved, except a small granular residue of acid tartrate of potassium, the presence of which is characteristic of wine vinegar. Vinegars made from malt or sugar leave a more or less glutinous residue very insoluble in alcohol. It is not uncommon for the grape juice to be fortified with sugar during fermentation or acetification. Vinegars resulting from this process will show a more or less glutinous insol-

uble residue when treated as above described. To definitely identify the cream of tartar, the crystalline residue should be separated by pouring off the alcohol and dissolving it in the minutest quantity of hot water. The liquid is cooled and the watch-glass on which it is placed is rubbed with a glass rod when the cream of tartar will be deposited in streaks along the track of the rod. Addition of a drop or two of alcohol renders the test more delicate.

The following figures are those of the Municipal Laboratory of Paris for white wine vinegars:—

	Sp. Gr.	Solids.	Sugar.	Potassium Bitartrate.	Ash.	Acetic Acid.
Maximum	1·0213	3·19	0·46	0·36	0·69	7·38
Minimum	1·0129	1·38	0·56	0·07	0·16	4·44
Mean	1·0175	1·93	0·22	0·17	0·32	6·55

It may be necessary to determine the amount of potassium bitartrate in a wine vinegar. This may be done by evaporating 25 c.c. of the vinegar to a syrupy consistency, and dissolving the residue in water to its original volume. One hundred c.c. of a mixture of ether and alcohol in equal parts are then added, and the mixture kept in a cold place for forty-eight hours. The precipitated tartrate is collected on a filter, washed with ether-alcohol, and finally dissolved in hot water and titrated with $\frac{N}{10}$ alkali. Each c.c. is equivalent to 0·0188 gr. of potassium bitartrate.

Fleury ("J. Pharm. Chim." 1910, 2, 264) considers that the presence of inositol is characteristic of wine vinegar (except distilled wine vinegar). He evaporates 100 c.c. nearly to dryness, takes up the residue with 50 c.c. of water, and adds 3 gr. of $\text{Ba}(\text{OH})_2$. The precipitate is separated centrifugally and washed with 30 c.c. of baryta water. The filtrate and washings are freed from excess of barium by a current of CO_2 . Ten c.c. of a 30 per cent solution of acetate of lead are added and the precipitate separated as before. The filtrate is made up to 100 c.c., and treated with 10 c.c. of basic lead acetate solution and 2 gr. of cadmium nitrate dissolved in water. The precipitate now formed contains the inositol. It is separated, decomposed by H_2S and the filtrate evaporated to a syrup, and taken up with 20 c.c. of absolute alcohol and 5 c.c. of ether. After standing for forty-eight hours, inositol, if present, separates in crystals, which are sweet and melt at about 250° .

Cider Vinegar.—This vinegar is of a pale yellow colour and has a distinct odour of the fruit. It is usually the weakest of all brewed vinegars, often containing as little as 3·5 per cent of acetic acid. The solid residue, which averages about 2 per cent, is somewhat mucilaginous, and during evaporation the odour of baked apples may be observed. The residue contains malic acid, but no tartaric acid.

The sugar, as indicated by reduction of copper solutions, should be the same before and after inversion (inversion should be accom-

plished by hydrochloric acid at 70° C.). Cider vinegar is optically active. If 25 c.c. of the vinegar be clarified by the addition of 2.5 c.c. of 10 per cent lead acetate solution, the filtered liquid should show an optical rotation between $-0^{\circ}6'$ and -4° in a 200 mm. tube. This vinegar is characterized by the presence of malic acid. If it does not give a precipitate with lead acetate which settles in a few minutes, it is not cider vinegar. To confirm the presence of malic acid, 5 c.c. of the vinegar is treated with 1 c.c. of a 10 per cent solution of calcium chloride. The liquid is filtered and to the filtrate about three times its volume of 95 per cent alcohol is added. In the presence of malic acid a flocculent precipitate will be formed. Dextrin will give a precipitate under these circumstances, but its presence will be indicated by a dextro-rotation in the polarimetric test. Sulphate will also yield a precipitate, although not of the same character. If the precipitate be collected, dried, dissolved in HNO_3 and evaporated to dryness on the water bath, malates will be converted into oxalates. The residue is treated with a little hot sodium carbonate solution, acidified with acetic acid, and tested with a solution of calcium sulphate. A precipitate of calcium oxalate is a decisive indication of malic acid.

Cider Vinegar, Analysis and Suggested Standards for.—A. E. Leach and H. C. Lythgoe ("Journ. Amer. Chem. Soc." xxvi. 375) recommend the following scheme of analysis of, and standards for, cider vinegar:—

Acetic Acid.—Three c.c. of vinegar are diluted with about 300 c.c. of water and titrated with $\text{N}/10\text{NaOH}$, using phenol-phthalein as indicator. The number of c.c. of alkali used, multiplied by 0.2 gives the percentage of acetic acid, which should not be less than 4.5 per cent. (This is incorrect: it may be under 3 per cent.)

Solids.—Five grms. of vinegar are weighed into a tared, flat-bottomed platinum dish, subjected for an hour to direct contact with the live steam of a boiling water bath, and the residue weighed. It should be approximately 2 per cent.

Ash.—The residue from the solids is carefully ignited in a muffle and the resulting ash weighed. It should be about 6 per cent of the solids. (It is often higher than this.)

Alkalinity of the Ash.—One hundred grms. of vinegar are evaporated to dryness in a platinum dish and the residue reduced to an ash in a muffle. The resulting ash is boiled with water, the solution filtered and the residue washed with boiling water till free from alkali. The filtrate is then treated with an excess of $\text{N}/10\text{HCl}$, the solution boiled to expel CO_2 , and the excess of acid titrated with $\text{N}/10\text{NaOH}$, using phenol-phthalein as indicator. The number of c.c. of $\text{N}/10\text{HCl}$ required for neutralization should be equivalent to at least 65 c.c. for each gm. of ash.

Soluble Phosphoric Acid.—The solution of the ash, after titration, is made acid with HCl , and evaporated to dryness, after which 50 c.c. of boiling H_2O are added, and the P_2O_5 determined by titration with uranium acetate in the usual way. At least 50 per cent of the total phosphates should be soluble in water.

Insoluble Phosphoric Acid.—The residue from the ash soluble in

water is dissolved in HCl, and the acid solution evaporated to dryness. The residue is then dissolved in about 10 drops of dilute HCl, 50 c.c. of boiling H_2O are added, then about 1 grm. of sodium acetate and the solution titrated with uranium acetate, as in the case of the soluble phosphoric acid.

Reducing Sugars.—Two portions of 25 c.c. each are measured into 100 c.c. flasks. One portion is diluted with 20 c.c. of water, 5 c.c. of concentrated hydrochloric acid are added and the solution subjected to inversion by heating to $70^\circ C.$ for ten minutes and cooling. Both portions are neutralized with sodium hydroxide and made up to a known volume. The reducing sugars determined by the ordinary methods should be the same before and after inversion. Any increase denotes the presence of cane sugar or glucose.

Polarization.—Twenty-five c.c. of the vinegar is precipitated with 2.5 c.c. of 10 per cent lead acetate solution, and filtered bright. It should show a rotation of between -0.1° to -4.0° in a 200 mm. tube.

Malic acid should be present, as shown both by the lead acetate and the $CaCl_2$ tests. If the vinegar under examination does not give a precipitate with lead acetate, settling in a few minutes, it is not cider vinegar. To confirm the presence of malic acid 5 c.c. of the vinegar is treated with 1 c.c. of 10 per cent $CaCl_2$ solution; filter and add to the filtrate about 3 volumes of alcohol 95 per cent. In the presence of malic acid a flocculent precipitate will occur. Dextrin is also thus precipitated by alcohol, but its presence will be indicated by a dextro-rotation on the polarimetric test. Sulphate also will give a precipitate of $CaSO_4$. If the alcohol precipitate be collected, dried, dissolved in HNO_3 , evaporated to dryness on the water bath, the calcium malate is converted into oxalate, which may be decomposed with Na_2CO_3 by boiling, acidified with HCl , and precipitated with $CaSO_4$. The last reagent is employed to prevent precipitation of any sulphates present as $CaSO_4$.

The value known as Winton's lead number (see under Vanilla) is sometimes determined on cider vinegars, as it often shows a considerable variation from the value obtained with malt vinegars. The average values are, according to Bailey, as follows:—

Cider vinegar	0.075 to 0.290
Malt vinegar	0.158 „ 0.548

Malt Vinegars.—The characteristics of malt vinegar are its comparatively high specific gravity, which averages above 1.020, its high extractives, containing much nitrogen and phosphoric acid. The water used for brewing malt vinegar often contains a high amount of sulphates and chlorides, so that no attention need be paid to reactions with barium chloride or silver nitrate.

Sugar Vinegar.—This vinegar is usually prepared from inverted starchy matter, glucose being the starting-point of the fermentation. This glucose vinegar generally contains dextrose, dextrin, and very frequently, calcium sulphate, in notable quantity. It therefore usually reduces Fehling's solution, and gives abundant precipitates with ammonium oxalate and barium chloride. To detect dextrin, the vine-

gar should be evaporated to about one-fifth of its bulk and then three to four times its volume of absolute alcohol added. Dextrin is precipitated in this manner. Dextrose remains in the filtrate, which is decolorized by boiling with animal charcoal, and the dextrose estimated by reduction of Fehling's solution, after the alcohol is boiled off. The optical rotation may be observed by concentrating the vinegar to half its bulk, clearing with lead acetate (see under cider vinegar) and filtering. It is usually, however, too small to give any decided results.

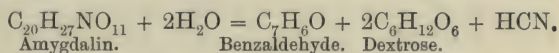
The above details will enable the analyst to differentiate, in general, between genuine brewed vinegars and diluted acetic acid coloured with caramel.

FLAVOURING ESSENCES.

Essence of Almonds.—Essence of almonds as sold to the public is a dilute alcoholic solution of essential oil of almonds, usually containing 1 to 2 per cent of the essential oil. The oil itself is used in pharmacy, but is not official in the British Pharmacopœia. Two varieties are recognized, one, the natural oil, the other deprived of its prussic acid, when it is then known as "Ol. Amygdalæ essent. S. A. P." (*sine acid prussic*).

Oil of Bitter Almonds.—The true bitter almond oil is obtained by distilling the seeds (almonds) of *Amygdalus communis* var. *amara* with water after the fixed oil has been extracted by expression. The kernels of the apricot and peach yield essential oils practically identical with that from the almond, and the greater part of the bitter almond oil of commerce, especially the foreign oil, is obtained from the former (*Prunus Armeniaca*). The following remarks may be taken as generally applicable to all three oils:—

Bitter almond oil does not exist as such ready formed in the seeds (almonds, kernels). It results from the action of water on the glucoside amygdalin, under the influence of the natural ferment emulsin present in the seeds. The reaction taking place is expressed by the following equation:—



Amygdalin, taking up two molecules of water, yields benzaldehyde, dextrose, and hydrocyanic acid. Amygdalin is a crystalline body, without any smell of the bitter almond, and does not yield the oil except under the influence of a hydrolytic agent, such as the natural ferment emulsin, or by boiling with dilute acids. The action of the ferment is destroyed by heat or by warm alcohol. Hence if dried and powdered bitter almonds are shaken with boiling water and distilled, no oil is obtained. After the fixed oil has been expressed the press-cakes are ground up and soaked for about twenty-four hours in twice their weight of water, to which a quantity of salt is usually added. The whole is then subjected to distillation. Some trouble, however, is experienced during the process, as the large quantity of

albuminoids present causes excessive frothing. To remedy this, the press-cakes are coarsely powdered and at once immersed in boiling water to coagulate the albuminoids and dissolve the amygdalin. The emulsin is, of course, rendered inactive, so that on cooling a quantity of emulsion of the fresh cake in cold water is added to the previously treated mass. This is allowed to stand, until the emulsin will have converted the whole of the amygdalin into essential oil. The mixture is now distilled. As hydrocyanic acid is a very deadly substance, it is necessary to use great care that none of the vapour is allowed to escape into the air. The oil of almonds so obtained contains a considerable amount of hydrocyanic acid, the remainder being principally benzaldehyde, $C_6H_5 \cdot COH$. The absolutely natural oil is a regular commercial article, but much is deprived of its hydrocyanic acid before being sold, and is then listed as "Oil of Almonds (S.A.P.)".

Oil of almonds also contains the nitrile of mandelic acid, and a trace of benzoin. Hydrocyanic acid is detected by adding to water which has been well shaken with the oil a little ferric and ferrous chloride or sulphate and solution of caustic potash. On acidifying the liquid a blue-green colour or precipitate is formed in the presence of hydrocyanic acid (ferro- and ferri-cyanides). It may be estimated by dissolving 1 grm. of the oil in 5 c.c. of alcohol and adding 50 c.c. of water. Ammonia silver nitrate solution is then added and the whole well shaken. The solution is then acidified with nitric acid and the silver cyanide washed, dried, and ignited.

The ignited precipitate corresponds to 25 per cent of its weight of HCN.

Natural almond oil is, in the crude state, a yellowish liquid, but is white when rectified, of specific gravity 1.045 to 1.070, but usually from 1.045 to 1.055. Its refractive index is about 1.5450, or if free from HCN, 1.550. It is optically inactive. It is often adulterated with artificial benzaldehyde, and if the purest variety be used it is impossible, within certain limits, to detect it, except, possibly by the nose. If the cheaper variety has been used, chlorine compounds will be present and may be detected as follows: A piece of filter paper is saturated with the oil and placed on a small porcelain dish standing in a larger one. A large beaker whose sides are moistened with distilled water is stood over the smaller dish, the paper having been set alight. The gases generated by the combustion are, to a certain extent, absorbed by the water on the sides of the beaker, which is rinsed out with a little more distilled water. The liquid is filtered, and one drop of nitric acid and a few drops of solution of silver nitrate are added to the filtrate; the formation of insoluble silver chloride is strong evidence that artificial benzaldehyde is present.

Another adulterant, of a much grosser character, is oil of mirbane. This is the cheap almond oil substitute so largely used for perfuming cheap toilet soaps. Chemically it is nitrobenzene, $C_6H_5NO_2$, more or less mixed with impurities, of which the most common is nitrotoluene, which sometimes itself forms the greater part of cheap nitrobenzene. Indeed, nitrotoluene in any great quantity may be regarded as an adulterant of nitrobenzene. The latter, when pure, is a yellowish

liquid of specific gravity at 0° of 1.200, boiling at about 206° , and solidifying at $+2^{\circ}$ to $+3^{\circ}$. It has a coarse almond-like odour, and is poisonous when taken internally, and irritating to the skin when used externally. The cheapness of benzaldehyde should discourage the use of mirbane in even the cheapest toilet soaps. Nitrotoluene, $C_6H_4(CH_3)NO_2$, exists in three isomeric modifications, and nitroxyline, $(C_6H_3)(CH_3)_2NO_2$, in more still. It is these bodies which are found to a considerable extent in the cheaper qualities of nitrobenzene. Consequently it is important that commercial samples should have physical characters in approximate agreement with those above quoted.

To detect the presence of this objectionable substituent in oil of almonds a little of the oil is warmed with iron filings and acetic acid. The nitrobenzene is reduced to aniline, $C_6H_5NH_2$, which is distilled off and collected. To the distillate a few drops of solution of ordinary chloride of lime is added. If aniline be present the liquid yields the characteristic violet colour. Pure benzaldehyde combines with sodium bisulphite to form a crystalline compound without the characteristic almond odour. Samples adulterated with nitrobenzene, when shaken with excess of bisulphite of sodium solution, so that the benzaldehyde is entirely combined, then have the characteristic coarse nitrobenzene odour.

To determine the benzaldehyde in diluted solutions of the oil such as ordinary essence of almonds, the following process (due to Denner, and elaborated by Denis and Dunbar) may be used:—

The reagents used are:—

Reagent (1):	Phenyl-hydrazine hydrochloride	. . .	2 grms.
	Crystals of sodium acetate	. . .	3 "
	Water	. . .	20 c.c.

Dissolve the sodium acetate in the water, add the phenyl-hydrazine hydrochloride, shake for five or six minutes, and filter, or

Reagent (2):	Phenyl-hydrazine	. . .	1 c.c.
	Glacial acetic acid	. . .	1.5 c.c.
	Water	. . .	20 c.c.

Mix the acetic acid and water, then pour in the phenyl-hydrazine.

Reagent (2) is much more convenient on account of the rapidity with which it may be prepared. Whether reagents (1) or (2) be used the solution should be made up immediately before use, and solutions more than an hour old should be discarded. The method of precipitation is as follows:—

Two 10 c.c. portions of almond essence are measured into 300 c.c. Erlenmeyer flasks; to one portion is added 10 c.c., to the other 15 c.c. of either reagent; shake, stopper tightly, and allow to stand over night in a dark place. The next day add 200 c.c. of cold water to each flask, and filter on tared Gooch crucibles provided with thin mats of asbestos. Wash with cold water and finally with a 10 c.c. of 10 per cent alcohol. Dry for three hours in a vacuum oven at 70° to 80° C. If a vacuum oven is not available, the drying may be accomplished in a vacuum desiccator over sulphuric acid, but will of

course take much longer than when a higher temperature is employed. The weight of the precipitate multiplied by 5.408 gives the number of grammes of benzaldehyde in 100 c.c. of the solution.

The reason for using 10 and 15 c.c. of reagent on different portions of the same extract is based on the fact that while the large majority contain in the neighbourhood of 1 per cent by volume of oil of bitter almonds, occasionally extracts are met with containing as much as 6 per cent of benzaldehyde; in such preparations it is obvious that while the use of 10 c.c. of the reagent may give good duplicates, the results would be far below the truth.

Several other processes have been suggested, especially a colorimetric process ("Journ. Amer. Chem. Soc." 1908, 1607), which the author finds gives fairly accurate results. It is carried out as follows:—

A solution of fuchsin-sulphurous acid is prepared fresh each time in the following manner: 0.5 gm. fuchsin is dissolved in water, and sulphurous acid introduced into the solution until the weight has increased by 20 grms. when it is further diluted to make 1 litre. In the next stage alcohol free from aldehyde is employed; this is obtained by taking spirit which has undergone a preliminary treatment with oxide of silver, and diluting it with 25 grms. phenylene-diamine hydrochloride per litre of alcohol, then passing a strong current of air through it for three hours, and distilling off, rejecting the first 100 c.c. A fresh standard solution of recently distilled benzaldehyde in aldehyde-free alcohol (1 mg. in 1 c.c.) is prepared. Ten grms. of the almond essence are then diluted to 50 c.c. with aldehyde-free alcohol; 2 c.c. of this solution is placed in a colorimeter tube and diluted to 20 c.c. Three control-solutions containing respectively 2, 4, and 6 mgs. benzaldehyde are then poured into tubes of equal size, all the tubes are brought to a temperature of 15°, the contents quickly diluted with 20 c.c. of the fuchsin-sulphurous acid solution. shaken up, and allowed to stand for ten minutes. As much of the sample-solution is now run off as will make its colour resemble that of one of the control-solutions. As the colour is in proportion to the degree of concentration of the benzaldehyde, the proportion of that body which is present may be calculated. The method gives accurate results, and is applicable also to bitter almond oil.

ESSENCE OF LEMON.

Essence of Lemon as sold to the public is usually an alcoholic solution of the essential oil of lemon, varying in strength from 5 to 30 per cent. The essential oil itself is frequently known under the same name.

Two varieties of essence of lemon are commonly met with; firstly that prepared by dissolving the essential oil in alcohol, secondly, that made by using terpeneless lemon oil—the essential oil freed from terpenes.

The examination of the essence of the shops is restricted to the separation and examination of the essential oil.

The oil is best separated by adding about 30 volumes of water to one of the essence, and allowing the liquids to completely separate in a separatory funnel, and, if necessary, dry the oil over a little fused acid potassium sulphate. It should then be examined as described under oil of lemon. The optical activity of the essence will indicate whether it has been made from ordinary oil of lemon or from the terpeneless oil, as the former has an optical rotation of about $+60^\circ$, and the latter, -7° , or thereabouts.

Essential Oil of Lemon.—This oil is official in the British Pharmacopœia which describes it as the oil obtained from fresh lemon peel. That authority requires it to have the following characters:—

Specific gravity, 0.857 to 0.860. Optical rotation not less than $+59^\circ$. The optical rotation of the first 10 per cent distilled should have a rotation not differing to more than 2° from that of the original oil.

These characters, especially the last, are seriously incorrect. Pure oils may have characters well outside these limits, especially in regard to the optical rotation of the first 10 per cent distilled. This depends so entirely on the form of the distillation flask and the rate of distillation, that very varying results can be obtained from the same oil. This is referred to later.

The well-defined hydrocarbons of lemon oil are the terpenes, limonene, lævo-pinene, camphene, and the sesquiterpene limene; limonene forming about 90 per cent of the oil.

The oxygenated bodies forming the other 10 per cent are citral, nonyl, and decyl aldehydes, geraniol and linalool and their acetic esters (the latter stated to be only present in Palermo oils, and thus probably accounting for the slight difference in odour between this and Messina oils), citraptene, $C_{11}H_{10}O_4$, and a stearoptene of unknown constitution.

Adulteration, which was until recently very frequent, is still common. Turpentine was the regular adulterant, with, at times, the poorer-quality distilled oil of lemons. But adulteration with turpentine is now so easily detected that the sophistication is frequently carried out in a more scientific manner.

Mixtures with the proper specific gravity and optical rotation can easily be made up from turpentine and orange oil—the poorer qualities of the latter of course being used—and such mixtures are often used to adulterate the oil.

But the most formidable adulterant from the analyst's point of view is one that has only come into vogue during the last few years, viz. the terpenes obtained in manufacturing the "terpeneless" or concentrated oil of lemon and oil of orange, the latter being sometimes added to turpentine to raise the optical rotation.

The terpenes are sometimes used alone, sometimes together with a little citral obtained from lemon-grass oil. Before discussing the analysis of lemon oil, a few words on the citral content will not be out of place. It is common custom to export oil of lemons with a guaranteed citral content, and to sell it upon that basis. In judging of the value of such a basis for market value of the oil, the following points should be carefully noted:—

1. The value of the oil depends on its percentage of oxygenated constituents, which are soluble in weak alcohol.

2. The terpenes are practically odourless and insoluble, therefore valueless for the general purposes for which lemon oil is employed.

3. The percentage of terpeneless oil obtained by careful fractionation is an indication of the value of the oil.

It would be more rational in valuing this oil not to give the percentage of citral (which might be, in fact often is, added as lemon-grass citral) but to return the amount of terpeneless oil actually obtainable by careful fractionation. It has been found in practice that genuine oils only yield from 5 to 6 per cent of terpeneless oil, which in the strict sense of the term are not terpeneless but contain a fair proportion of sesquiterpenes; however, such oils will not contain more than 50 per cent of total aldehydes, and therefore the amount of citral calculated on the original oil would be 4 per cent. No lemon oil ever contains anything like 7 per cent of citral, which is a figure given by Messina analysts.

The specific gravity of a pure lemon oil should be between 857 and 862. These limits are rarely exceeded. The optical rotation taken at 20° should not be less than + 57°, and is usually between this figure and + 64°. Increase in temperature causes a slight diminution in rotatory power, but this does not amount to more than about - 8' or - 9' per degree rise in the temperature of the oil. The refractive index for pure oils lies within the comparatively narrow limits of 1.4748 to 1.4754. Pure lemon oil commences to boil at 170° to 172° and from 20 to 30 per cent will be obtained from 172° to 174°. However, the percentage of the fractions at temperatures near to one another are so dependent on the exact form of the distillation flask that the results are not constant enough to be of much value unless any oil distils below 170°.

To determine the purity or otherwise of lemon oil a somewhat prolonged analysis is necessary, no one constant being of much value by itself.

The following scheme will, however, detect any adulteration:—

1. Determine the specific gravity at 15° C.

2. Optical rotation (100 mm. tube).

3. Refractive index, at 20° C.

4. Fractionally distil as follows:—

Introduce into a distilling flask having three bulbs blown in the neck 100 c.c. of the oil to be tested. The receiver is an ordinary Bruhl apparatus with two vessels graduated at 10 and 80 c.c. respectively. The whole apparatus is then exhausted by means of a water pump (or other suitable means), and when a pressure of not more than 20 mm. is obtained, as shown on a gauge, the distillation is commenced by gently heating the oil-bath containing the flask.

The first fraction is collected in the 10 c.c. tube and the second in the 80 c.c. flask. Directly the second fraction is collected the pressure is released, and the distillation continued by passing a current of steam into the distillation flask and collecting the oil and water into a suitable vessel. When about 200 c.c. of water have been

collected the distillation is stopped; the oil is then separated from the water and carefully measured.

The rotation and refractive index of the three fractions are then carefully determined, and further with No. 3 the aldehydes are estimated by absorption with acid sulphite of soda.

Interpretation of results:—

Fraction 1 will indicate the addition of turpentine; there should not be a greater difference than 8° between the rotation of this and of the original oil, 5° to 6° being the average for normal oils.

Note.—Pinene is a natural constituent of lemon oil, but only in traces.

The refractive index will be about 2 points in the third place of decimals lower than that of the original oil. This fraction will also indicate substances of low boiling-point.

Fraction 2 will indicate chiefly the addition of added terpenes, normal oils showing an *increase* of 6° to 7° rotation from the original oil, whereas with added terpenes the increase will be considerably higher and the refractive index lower.

Fraction 3 is in many respects the most important, inasmuch that it indicates the true proportion of oxygenated constituents of the oil and therefore the strength of the oil. The rotation will depend partly on the amount of oil obtained by the steam distillation which is usually between 6.5 to 7.5 c.c., the rotation being about $+2^\circ$ to $+14^\circ$, but in some very rich oils the sign may be minus.

The refractive index will also have increased to nearly 1.4800 in this fraction. The aldehydic content should be in direct ratio to the amount of the fraction from 36 to 46 per cent.

It must be remembered that this method does not give the actual amount of citral present in the oil, but only that obtainable in the only practicable method of manufacturing terpeneless oil of lemon. There are many methods of determining citral suggested, none of which are very satisfactory. Of those, the following gives the most exact results:—

The analysis is conducted as follows:—

Twenty c.c. of lemon oil are mixed with 20 c.c. of $\frac{N}{2}$ solution of hydroxylamine hydrochloride in 80 per cent alcohol, and to the mixture is added about 8 c.c. of $\frac{N}{1}$ alcoholic potash and 20 c.c. of strong alcohol (which is sufficient to procure complete solution when hot).

The mixture is boiled gently under a reflux condenser for half an hour, and then allowed to cool. The condenser is washed down, and the contents of the flask diluted with about 250 c.c. of water, and neutralized to phenol-phthalein. The liquid is then titrated with $\frac{N}{2}$ sulphuric acid, using methyl orange as indicator. The number of c.c. of acid required, subtracted from the number used in a blank experiment, in which no lemon oil is present, gives the amount of hydroxylamine which has entered into reaction with the citral, and multiplied by 0.076 gives the weight of citral.

When the titration of methyl orange is performed in the usual way with addition of a drop of the indicator to the solution, the end point is often not very satisfactory. Much sharper results are obtained by making use of drops of a very dilute aqueous solution of methyl orange scattered on a white plate. When drops of the solution which is being titrated are brought into contact with these the change of colour when neutralization is complete is well marked.

Hiltner proposes ("J. Ind. Eng. Chem." 1909, **1**, 798-800, through "J. Soc. Chem. Ind." 1910, **29**, 172), a method of citral determination based on the fact that a 1 per cent solution of m-phenylenediamine hydrochloride in 50 per cent alcohol gives a clear yellow coloration with citral at the ordinary temperature. The determination is performed in the usual manner, the solution being made up to volume with 90 to 95 per cent alcohol in the case of lemon extracts and with 50 to 60 per cent alcohol in the case of terpeneless extracts. He claims that this method is more accurate than the magenta sulphurous acid process since under the experimental conditions the reagent gives no coloration with acetaldehyde or citronellal. The method, however, fails in the case of lemon oil which has become altered by oxidation, the coloration produced varying from yellowish-green to greenish-blue, according to the degree of oxidation of the oil.

Chace ("U. S. Dept. of Agriculture, Bureau of Chemistry," Circular No. 46, 1909) has published the following method, which he claims will detect traces of pinene in lemon oil, and thus indicate adulteration with turpentine.

Fifty c.c. of the oil is distilled and is then tested for pinene as follows:—

The distillate is mixed with an equal volume of glacial acetic acid in a 2-ounce Erlenmeyer flask, and immersed in a freezing mixture. Ten cubic centimetres of ethyl nitrite are next added and finally, slowly with constant stirring, 2 c.c. of a mixture of two parts of concentrated hydrochloric acid and 1 part of water, all previously cooled. The whole is allowed to remain fifteen minutes in the freezing bath, then rapidly filtered on a Gooch crucible provided with a filter paper disc, using a vacuum. The resulting crystals of nitroso-chloride of limonene are dissolved in the smallest possible amount of chloroform and reprecipitated with methyl alcohol. After filtering off these crystals, they are mounted with olive oil and examined under the microscope, using a magnification of 100. (See Fig. 34.) If present, pinene nitroso-chloride is easily detected by its comparatively broad crystals having irregular pyramidal ends, limonene nitroso-chloride crystallizing in needle shapes or columns.

This method has been very severely criticized by the author, and the consensus of opinion is that a positive reaction cannot be taken as any proof of adulteration of lemon oil, on account of the small, varying amount of pinene naturally present in lemon oil.

Further, the published results of such authorities as Wallach, in reference to the crystallographic characters of the nitroso-chlorides, do not support the contentions raised by Chace.

For fuller details of this subject "The Chemistry of Essential Oils" (by the author) should be consulted.

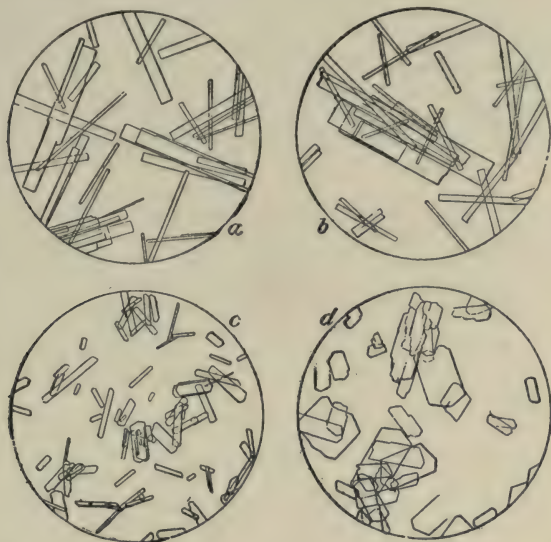


FIG. 34.—Photomicrograph of crystals from lemon oil ($\times 100$). *a, b*, Limonene nitroso-chloride crystals from lemon oil; *c*, limonene and pinene nitroso-chloride crystals from a lemon oil mixed with 5 per cent of turpentine; *d*, pinene nitroso-chloride crystals from turpentine.

VANILLA.

Vanilla is the fruit of an orchid, *Vanilla planifolia*, and other closely allied species, grown chiefly in Mexico, Bourbon, Tahiti, and the Seychelles. The fruits are long pods varying in length from about 3 to 8 or 9 inches, and differing materially in odour according to the country in which they are grown.

Vanilla owes its value chiefly to the presence of the odorous body vanillin—small quantities of other odorous bodies, of course, being present.

The fruits, or beans, as they are called, are judged by their appearance and odour, and do not often come, as such, before the analyst. The only forms of adulteration possible are the addition of exhausted beans, or the apparent improvement in appearance of inferior beans by coating them with crystals—usually of benzoic acid. The most valued kinds of vanilla are incrustated with fine crystals of vanillin—which gradually find their way to the surface of the bean from the interior.

The following analyses of vanilla beans are due to König:—

	1.	2.
	Per cent	Per cent
Water	25.85	30.94
Nitrogenous matter	4.87	2.56
Fat and wax	6.74	4.68
Reducing sugars	7.07	9.12
Non-nitrogenous extractives	30.50	32.90
Cellulose	19.60	15.27
Ash	4.73	4.53

Vanillin is a methyl-protocatechuic aldehyde, $C_6H_3(COH)(OCH_3)(OH)$, forming small white crystals melting at 81° to 82° , and possessing an intense vanilla odour. It is now produced synthetically in enormous quantities, and has largely replaced the natural vanilla bean as a flavour. A very large amount of the chocolate of commerce is flavoured with artificial vanillin. It is prepared in many different ways, the principal of which is by the oxidation of eugenol, the principal constituent of oil of cloves.

The eugenol is first acetylated by means of acetic anhydride, and the resulting acet-eugenol is dissolved in acetic acid and oxidized with permanganate of potassium. The liquid is then filtered, and rendered alkaline, and the whole is then evaporated, and the residue treated with moderately dilute acid, and extracted with ether. The ethereal solution is extracted with a solution of sodium bisulphite, which combines with the vanillin. The double sulphite compound is decomposed with dilute sulphuric acid, and the vanillin is extracted with ether, from which solvent it is obtained in fine white crystals.

The best yield, however, is obtained by first converting the eugenol into iso-eugenol $OH.OCH_3.C_6H_3.CH:CH.CH_3$ by treating it with solution of potassium hydrate. The acetylation product is oxidized, by which acetyl-vanillin is chiefly formed, which yields vanillin by splitting off the acetyl group.

Some of the cheaper commercial samples are heavily adulterated with acetanilide. The effect of this body is to lower the melting-point even if present in large quantity, but it is very easily detected, as by boiling with solution of potash, aniline is formed, which is easily detected by any of the usual reactions. A quantitative separation may be effected as follows: the substance is dissolved in ether and the liquid repeatedly shaken with concentrated solution of sodium bisulphite. The vanillin is thus extracted, and the ether, after being washed twice with water, is allowed to evaporate, when the acetanilide remains. This will then be found to have a melting-point close to 113° . Benzoic acid and coumarin are also occasional adulterants of vanillin. A little isovanillin $C_6H_3(CHO)^1(OH)^3(OCH_3)^4$ is occasionally present, but this is due to the fact that it is generally formed in small quantity with vanillin, in many reactions.

Sugar is occasionally found as an adulterant, but is easily detected by its insolubility in ether: after extraction with the vanillin it can be dissolved in water and recognized by any of the usual tests.

Acet-iso-eugenol, one of the intermediate bodies in the manufacture of vanillin is sometimes found; it lowers the melting-point of the sample, yields acetic acid in hydrolysis, and gives a fine red colour with strong sulphuric acid, whereas pure vanillin only gives a lemon-yellow colour. Benzoic acid is also found as an adulterant. This is easily detected by the high acid value of the substance (vanillin is neutral), and by dissolving the sample in ether, extracting the vanillin by means of sodium bisulphite solution, and neutralizing the residue from the ethereal solution with potash, dissolving it in water, and testing it with a neutral solution of ferric chloride, when red ferric benzoate is precipitated.

In examining vanilla beans the determination of the vanillin is a matter of importance. Busse recommends the following process for the determination: 20 grms. of the pods, crushed with sand, are exhausted with ether in a Soxhlet tube, and the ethereal extract is shaken out with 20 per cent sodium bisulphite solution. From the latter, vanillin is removed by treatment with dilute H_2SO_4 , the SO_2 generated removed by a current of CO_2 , and the vanillin extracted by shaking out with ether, evaporating the solvent and weighing the residue. In East African vanilla the author found 2.16 per cent of vanillin, in that from Ceylon 1.48 per cent, and in Tahiti vanilla from 1.55 to 2.02 per cent. Tiemann and Haarman found in the best Bourbon vanilla 1.94 to 2.90 per cent, in the best Java vanilla 2.75 per cent and in Mexican vanilla from 1.7 to 1.9 per cent. Tahiti vanilla sometimes contains less than 1 per cent of vanilla.

In suspected cases the crystals on the beans should be carefully separated and examined for benzoic acid as above described.

Hanus ("Analyst," xxv. 318) recommends that β -naphthyl hydrazine hydrochloride should be added to the solution of vanillin in such proportion that from two to three parts are present for each part of vanillin. After standing for five hours the precipitate is transferred to a tared filter, washed with hot water until the washings no longer precipitate silver nitrate, dried at 90° and weighed. The weight of the hydrazine formed, divided by 1.92 gives that of the vanillin present. This method is available in all cases where an aqueous solution of the vanillin can be prepared.

Hanus has more recently recommended the following method for the determination of vanillin in vanilla beans and in preparations thereof ("Pharm. Zeit." 50, 1022, 157). Three grms. of the crushed pods are extracted for three hours in a Soxhlet tube with ether, the solvent distilled off cautiously, and the residue dissolved in a little ether, the solution filtered and the filtrate evaporated cautiously. The residue is treated with 50 c.c. of water at 60° on a water bath: 0.25 gm. of *meta*-nitrobenzhydrazide is then added to the aqueous solution in a stoppered flask, which is kept for two to three hours at 60° , and then set aside with occasional shaking for twenty-four hours. The vanillin is precipitated quantitatively as vanillin-*meta*-nitrobenzhydrazone, $\text{NO}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{NH}_4 \cdot \text{N} : \text{CH} \cdot \text{C}_6\text{H}_3(\text{OCH}_3) \cdot \text{OH}$. The precipitate is washed with three successive quantities of petroleum ether to remove fat, then washed with water, and then again with petroleum

ether, and then dried at 100° for two hours. The weight, multiplied by 0.4829 gives the amount of vanillin present. Preparations of vanillin are treated similarly, alcohol being removed by evaporation. The presence of other aldehydes, such as heliotropin, of course, will vitiate the results.

Essence of Vanilla.—The substance sold under this name is, properly, a spirituous extract of the vanilla bean. Many samples, however, are little more than alcoholic solutions of artificial vanillin, coloured with caramel. Some samples, which cannot be described as adulterated, contain a little coumarin or other odorous substance, added to vary the characteristic vanillin odour and flavour somewhat.

A genuine extract can be recognized by the fact that it contains some dark red or red-brown resin, soluble in 50 per cent alcohol, but precipitated on further dilution.

Coumarin, or extract of Tonka beans, which contain coumarin, may be detected as follows: a small quantity of the essence is evaporated to dryness, the residue fused with caustic potash, saturated with hydrochloric acid and treated with a drop of ferric chloride solution. If coumarin be present, a violet colour, due to the formation of salicylic acid, will be produced.

Winton and Silverman ("Jour. Amer. Chem. Soc." **24**, 1128) recommend the following methods for examining essence of vanilla:—

De-alcoholize 25 grms. of the extract in an evaporating dish upon a water bath, at a temperature of about 80° C., adding water from time to time to retain the original volume. After removal of the alcohol, add normal lead acetate solution, drop by drop, until no more precipitate forms. Stir to facilitate flocculation of the precipitate, filter through a moistened filter, and wash three times with a few c.c. of hot water. Cool the filtrate and extract with ether by shaking out in a separator. Use 15 c.c. to 20 c.c. of ether at each separation, repeating the process three or four times, or until a few drops of the ether, evaporated upon a watch glass, leaves no residue. Place the combined ether extracts containing all of the vanillin and coumarin in a clean separator, and shake out four or five times with 2 per cent ammonia, using 10 c.c. for the first, and 5 c.c. for each subsequent shaking.

Set aside the combined ammoniacal solutions for the determination of vanillin.

Wash the ether solution into a weighed dish, and allow it to evaporate at the room temperature. Dry in a desiccator and weigh. Usually the dried residue is pure coumarin. Treat the residue with 5 c.c. to 10 c.c. of cold petroleum ether, boiling between 30° C. and 40° C., and decant off the clear liquid into a beaker. Repeat the extraction with petroleum ether until a drop, evaporated on a watch glass, leaves no residue. Dry the dish for a few moments in a water oven, cool and weigh. Subtract the weight of the dish and the residue (if any) from the weight previously obtained after evaporation with ether, thus obtaining the weight of pure coumarin. Allow the petroleum ether to evaporate at the room temperature, and dry, if necessary, in a desiccator. The residue should be crystalline and have a melting-point of

67° C. This, with the characteristic odour of coumarin, is sufficient for its identification.

Slightly acidulate the reserved ammoniacal solution of vanillin with 10 per cent hydrochloric acid. Cool and shake out in a separatory funnel with four portions of ether of about 15 c.c. to 20 c.c. each. Evaporate the ether at room temperature in a weighed platinum dish, dry over sulphuric acid, and weigh. Treat the residue with boiling petroleum ether (boiling-point 80°), decanting into a dry beaker. Repeat the treatment until all vanillin is removed. Dry the dish and residue (if any) for a few moments at 100° C. and weigh; deduct the weight from the weight of the ether residue. The difference is the weight of the vanillin. Evaporate the petroleum ether at ordinary temperatures, and dry in a desiccator. The residue should be crystalline, and melt at 80° C. to 81° C.

Tests for Caramel.—Valuable indications of the nature of an extract are obtained in the process of determination of vanillin and coumarin. Pure extracts of vanilla beans give, with lead acetate, a bulky, more or less glutinous, brown-grey precipitate, and a yellow or straw-coloured filtrate, whereas purely artificial extracts coloured with caramel give a slight dark brown precipitate and a dark brown filtrate. If both vanilla bean extract and caramel are present the precipitate is more or less bulky and dark-coloured, and the filtrate is more or less brown. The solution remaining after extraction of the vanillin and coumarin with ether, if dark-coloured, should be tested for caramel.

The most satisfactory test for caramel is to shake with fuller's earth, as recommended by Crampton and Simons. If the colour is due to caramel and a grade of fuller's earth is used, which experience has proved suitable, the solution, after filtering, is yellow or colourless. This test does not positively identify the colour, as some other brown substances may give similar reactions, but no such substance is liable to be present in vanilla extract.

Winton and Bailey determine vanillin, coumarin and acetanilide (which is sometimes found as an adulterant of artificial vanillin, and therefore indicates its presence), in the following manner, which is a modification of the method devised by Hess and Prescott ("Jour. Amer. Chem. Soc." 1899, 256):—

Twenty-five grms. of the essence are weighed into a 200 c.c. beaker, marked to indicate volumes of 25 c.c. and 50 c.c. The essence is diluted with water to 50 c.c. and evaporated on a water bath to 25 c.c. at a temperature not exceeding 70°. It is now again diluted to 50 c.c., and evaporated to 25 c.c. Solution of acetate of lead is then added until no further precipitation takes place. The liquid is then, after being well stirred, filtered through a moistened filter paper, and washed three times with hot water, so that the total filtrate does not exceed 50 c.c. The filtrate, when cold, is shaken with 20 c.c. of ether in a separator. The ether is separated, and the liquid extracted with three further portions of 15 c.c. of ether. The combined ether extracts are then shaken with 10 c.c. of 2 per cent ammonia solution and with three subsequent portions of 5 c.c. The

etheral solution is reserved (B) and the combined ammoniacal solutions are rendered slightly acid with 10 per cent hydrochloric acid. The liquid is then extracted four times with ether, and the ether evaporated and the residue dried at room temperature, and finally in a desiccator and weighed (A). If acetanilide is absent, this may be taken as pure vanillin, which should melt at 79° to 81° . If acetanilide has been detected (*vide infra*), the residue should be dissolved in 15 c.c. of 10 per cent ammonia, and the liquid shaken twice with ether. The ether, on evaporation, will leave a residue of acetanilide, which is dried at room temperature and then in a desiccator and the weight deducted from the "vanillin" (A) previously weighed. The total amount of acetanilide is the amount thus obtained, together with that present in the ethereal solution (B) reserved above. The latter is transferred to a tared dish and the ether allowed to evaporate at room temperature. The residue is dried in a desiccator and weighed. It is then extracted several times by stirring well with petroleum ether, which is decanted each time. If the residue is thus completely dissolved, it may be taken to be entirely coumarin. Any undissolved residue is probably acetanilide (melting-point 112° to 113°) and its weight deducted from the total residue gives the coumarin.

The acetanilide here found is added to the amount extracted with the vanillin to give the total amount present.

The presence of acetanilide in these residues may be confirmed by boiling the residue for two to three minutes with HCl, and when cool, adding a few drops of 0.5 per cent of chlorinated lime solution, in such a manner that the liquids do not mix. A fine blue colour results if acetanilide be present.

Commercial essence of vanilla is usually made with about 5 per cent of vanillas, the menstruum varying in strength from 40 to 50 per cent alcohol in the best varieties. Sugar is sometimes added, but not always. The average vanillin content is 0.1 to 0.2. Much higher values than these indicate the presence of synthetic vanillin.

Jackson and McGeorge have determined the lead number (see p. 272) of vanillas (calculated to 100 grms. of the beans) and consider that for a given bean, this value is constant irrespective of the strength of the alcohol used to extract the vanillas, so long as not more than 10 per cent of bean be used for the essence.

The table at top of opposite page represents their analytical results.

It is thus noticed that the "Lead Number" is practically a constant for any particular bean regardless of the strength of alcohol used in the percolation, which would indicate that practically all of the bodies precipitated by basic lead acetate are removed by any strength of alcohol likely to be used, and that the "Lead Numbers" could be used as a measure of the quantity of vanilla beans used in the manufacture of an extract.

Bean.	Per cent Alcohol.	Lead Number.	Duplicate.
Prime :			
Mexican	65	1.62	—
Mexican :			
Cuts	50	1.82	—
"	50	1.91	—
"	50	1.90	—
Prime :			
Mexican	65	1.60	—
"	20	1.64	1.63
"	30	1.68	1.68
"	40	1.66	1.65
"	65	1.65	1.65
Ordinary :			
Mexican	20	1.80	1.82
"	30	1.82	1.80
"	40	1.81	1.81
"	65	1.83	1.80
Mexican :			
Cuts	20	2.00	2.00
"	30	2.03	2.01
"	40	2.10	2.09
Bourbon	20	1.56	1.55
"	30	1.59	1.59
"	40	1.68	1.68
"	65	1.60	1.60
South American	20	1.62	1.62
" "	30	1.42	1.42
" "	40	1.48	1.47
" "	65	1.51	1.49
Vanillons	20	1.02	1.02

To test this point three extracts were made up as before, but with 5, 10 and 15 grms. of beans to each 100 c.c. The results follow:—

LEAD NUMBERS.

Bean.	Experiment.	Per cent Alcohol.	Lead Number.	Duplicate.	Grms. in 100 c.c.
Bourbon	A	65	0.84	0.83	5
"	B	65	1.68	1.68	10
"	C	65	1.79	1.80	15

Experiments A, B and C seem to indicate that the "Lead Number" is a measure of the quantity of beans used when this does not exceed 10 grms. in 100 c.c.

Although, as indicated above, there is no standard in this country for essence of vanilla, it may be regarded as certain that, if an essence gives no precipitate with a solution of lead acetate, it is made entirely from artificial vanillin, and contains no natural vanilla at all.

Winton and Lott ("U.S. Dept. Agric. Bull.," 132, 1910) state that if normal lead acetate solution be used instead of the basic acetate the

lead numbers are lower. For artificial essences, they find values between 0.03 and 0.17; for natural essences, between 0.29 and 0.34, but the last-named figures must depend entirely on the strength of the essence. To determine the lead number they dilute 50 gr. of essence to 80 c.c. with water, evaporate to 50 c.c., add 30 c.c. of water, again evaporate to 50 c.c. and add 25 c.c. of an 8 per cent normal lead acetate solution. The whole is diluted to 100 c.c., allowed to stand several hours and filtered. To 10 c.c. of the filtrate, 25 c.c. of water, excess of sulphuric acid and 100 c.c. of 95 per cent alcohol are added. After sixteen hours the PbSO_4 is collected and weighed. The lead number is given by the formula

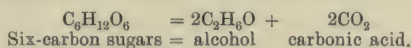
$$P = \frac{100 \times 0.6831(S - W)}{5} = 13.662(S - W)$$

where P is the lead number, S = gr. of PbSO_4 obtained from 2.5 c.c. of the lead acetate solution, and W the weight of PbSO_4 obtained from 10 c.c. of the filtrate.

CHAPTER V.

ALCOHOLIC BEVERAGES.

BEFORE dealing with spirits, wines, and beer, it is necessary to deal shortly with pure alcohol, which, of course, is never sold in the ordinary way as a beverage, but is official in the British Pharmacopœia. All alcoholic beverages may be classed as products of fermentation of saccharine matter. In the case of fruits, sugars exist ready formed in their juices, and any expressed fruit juice will rapidly commence to ferment when left to itself, since the microscopic organisms responsible for the process are universally diffused. In the cases of beverages made from cereals the first process is the conversion of the starchy matter of the grain into sugars, by the action of an unorganized ferment such as diastase, the active ferment of malt. In the case of beers, sugars are often added to a mixture of malted and unmalted grain, and in this country no objection is taken to such additions. The manufacture of beers is under much greater control than is the case with wines, so far as the fermentation process is concerned, since in the former case there is a good deal of selected yeast used, whilst in the latter, the yeasts mostly find their way into the fruit juice, either from the air or from the skins of the grapes. The greater part of the sugar present in fruits, especially in the grape, is invert sugar, which is, of course, capable of direct fermentation. The theoretical reaction usually expressed as representing ordinary fermentation processes is as follows:—



As a matter of fact, however, one obtains no more than 48·5 per cent of alcohol and 46·5 per cent of carbonic acid, the remaining 5 per cent being glycerin, succinic acid and traces of higher alcohols and esters, which may be termed the secondary products of alcoholic fermentation.

Alcohol.—Alcohol, or ethyl alcohol $\text{C}_2\text{H}_5\text{OH}$, is, chemically, methylcarbinol. It is a colourless liquid with a pleasant odour, boiling at $78\cdot3^\circ$, and of specific gravity $0\cdot7939$ at $15\cdot5^\circ$. Alcohol and water are miscible in all proportions with evolution of heat and a contraction in volume. Alcohol obtained in as strong a form as can be made by ordinary distillation is known as rectified spirits of wine, or “rectified spirit,” the latter being the official name of the British Pharmacopœia, which requires it to have a specific gravity $0\cdot8340$ and to contain 85·65 per cent of alcohol by weight (= 90 per cent by volume).

Proof spirit is an excise term, having its origin in the fact that in olden days, the excise tested alcohol by pouring it on to a weighed quantity of gunpowder. If it was above a certain strength, the gunpowder ultimately exploded when a light was applied, but if it was below that strength, the powder was too saturated with moisture to ignite. Hence the terms "over proof" and "under proof". Proof spirit is now defined by statute to mean of such density that at 51° F. 13 volumes shall weigh the same as 12 volumes of water. This spirit has a specific gravity at 15.5 C. of 0.9198 and contains 49.24 per cent of alcohol by weight or 57.06 per cent by volume. The expression "degrees under proof" and "degrees over proof" are very confusing and rarely understood out of this country. If a liquid is 20° under proof it is meant that it contains 80 volumes of proof spirit and 20 volumes of water. If a liquid is spoken of as being 20° over proof it contains so much alcohol that if a 100 volumes be diluted with water to 120 volumes it will be of proof strength. Absolute alcohol is 75.25° over proof. As nearly every determination of alcohol is made by taking the specific gravity of the liquid containing (practically) nothing but alcohol and water, it is necessary to have a table of reference from which the values can at once be taken. A very elaborate table is in the course of preparation by the excise authorities at the Government laboratories, but is not available at the time of going to press. The table on following pages is of a high degree of accuracy and gives the percentage of alcohol by weight and by volume, and also the degrees under and over proof of all mixtures of alcohol and water, of specific gravities between 0.7938 and 1.000, with differences of 0.0005 throughout:—

Methylated spirit is alcohol suitably denatured so as to be unfit for human consumption. In this country two varieties of methylated spirit are known, both being allowed to be sold without an excise duty. Of these, that which is used solely for manufacturing purposes on the premises to which it has been delivered is of much less objectionable odour than that which is resold from such premises. Wood naphtha is the principal denaturant so that it is a matter of importance to examine certain alcoholic preparations for methyl alcohol, which would indicate the illegitimate use of duty free spirit.

The detection of alcohol is not a difficult matter, and is a question of some importance in the examination of temperance beverages. The following are the principal reliable tests for the detection of alcohol:—

(1) The iodoform test. This test will detect from 0.1 per cent to 0.2 per cent of alcohol. The liquid is warmed with a few drops of concentrated solution of iodine in potassium iodide, and then enough solution of caustic soda to nearly decolorize. The characteristic odour of iodoform will be developed, and on standing a slight yellow precipitate of iodoform appears: if the amount exceeds 0.3 per cent to 0.4 per cent the precipitate is formed quickly. The iodoform crystals, when examined with a powerful hand lens, will often show their crystalline structure, and under the microscope they may be seen as star-shaped groups or six-sided tablets. It must be remembered that several other substances such as acetone and lactic acid yield this reaction.

ALCOHOL TABLE.

Under Proof.

Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent <i>under</i> Proof.	Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent <i>under</i> Proof.
1·000	0·00	0·00	0·00	·9775	15·25	18·78	67·10
·9995	0·26	0·31	99·43	·9770	15·67	19·28	66·20
·9990	0·55	0·65	98·80	·9765	16·08	19·78	65·34
·9985	0·80	1·00	98·24	·9760	16·46	20·24	64·53
·9980	1·05	1·30	97·62	·9755	16·85	20·71	63·72
·9975	1·34	1·65	97·05	·9750	17·25	21·19	62·87
·9970	1·60	2·00	96·46	·9745	17·67	21·69	62·00
·9965	1·89	2·35	95·84	·9740	18·08	22·18	61·13
·9960	2·15	2·70	95·23	·9735	18·46	22·64	60·32
·9955	2·45	3·10	94·62	·9730	18·85	23·10	59·52
·9950	2·75	3·50	93·98	·9725	19·25	23·58	58·67
·9945	3·02	3·82	93·36	·9720	19·67	24·08	57·80
·9940	3·30	4·15	92·70	·9715	20·08	24·58	56·93
·9935	3·61	4·52	92·08	·9710	20·50	25·07	56·06
·9930	3·90	4·90	91·45	·9705	20·92	25·57	55·20
·9925	4·20	5·27	90·80	·9700	21·31	26·04	54·37
·9920	4·50	5·65	90·12	·9695	21·69	26·49	53·57
·9915	4·82	6·02	89·45	·9690	22·08	26·95	52·77
·9910	5·15	6·40	88·78	·9685	22·46	27·40	51·98
·9905	5·45	6·78	88·08	·9680	22·85	27·86	51·18
·9900	5·75	7·15	87·40	·9675	23·23	28·31	50·38
·9895	6·09	7·42	86·70	·9670	23·62	28·77	49·60
·9890	6·40	8·00	85·98	·9665	24·00	29·22	48·80
·9885	6·75	8·40	85·27	·9660	24·38	29·67	48·00
·9880	7·10	8·80	84·53	·9655	24·77	30·13	47·20
·9875	7·43	9·22	83·80	·9650	25·14	30·57	46·44
·9870	7·80	9·65	83·05	·9645	25·50	30·98	45·70
·9865	8·13	10·10	82·30	·9640	25·86	31·40	44·97
·9860	8·50	10·55	81·54	·9635	26·20	31·80	44·27
·9855	8·84	10·97	80·77	·9630	26·53	32·19	43·60
·9850	9·20	11·40	79·99	·9625	26·87	32·58	42·90
·9845	9·56	11·87	79·22	·9620	27·21	32·98	42·20
·9840	9·90	12·35	78·45	·9615	27·57	33·39	41·47
·9835	10·35	12·77	77·53	·9610	27·93	33·81	40·74
·9830	10·81	13·20	76·53	·9605	28·25	34·18	40·10
·9825	11·23	13·65	75·64	·9600	28·56	34·54	39·47
·9820	11·45	14·10	74·83	·9595	28·87	34·90	38·84
·9815	12·00	14·60	74·00	·9590	29·20	35·28	38·18
·9810	12·25	15·10	73·18	·9585	29·53	35·66	37·50
·9805	12·77	15·65	72·36	·9580	29·87	36·04	36·83
·9800	13·00	16·00	71·54	·9575	30·17	36·39	36·23
·9795	13·54	16·70	70·73	·9570	30·44	36·70	35·68
·9790	13·92	17·17	69·90	·9565	30·72	37·02	35·13
·9785	14·36	17·70	68·97	·9560	31·00	37·34	34·57
·9780	14·82	18·25	68·00	·9555	31·31	37·69	33·95

ALCOHOL TABLE.

Under Proof.

Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent <i>under</i> Proof.	Sp. Gr. at 60° C.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent <i>under</i> Proof.
·9550	31·62	38·04	33·32	·9370	41·30	48·75	14·57
·9545	31·94	38·40	32·70	·9365	41·55	49·02	14·10
·9540	32·25	38·75	32·08	·9360	41·80	49·29	13·63
·9535	32·56	39·11	31·46	·9355	42·05	49·55	13·16
·9530	32·87	39·47	30·84	·9350	42·29	49·81	12·70
·9525	33·18	39·81	30·24	·9345	42·52	50·06	12·27
·9520	33·47	40·14	29·66	·9340	42·76	50·31	11·82
·9515	33·76	40·47	29·08	·9335	43·00	50·57	11·38
·9510	34·05	40·79	28·52	·9330	43·24	50·82	10·94
·9505	34·29	41·05	28·06	·9325	43·48	51·07	10·50
·9500	34·52	41·32	27·60	·9320	43·71	51·32	10·05
·9495	34·76	41·58	27·13	·9315	43·95	51·58	9·60
·9490	35·00	41·84	26·67	·9310	44·18	51·82	9·20
·9485	35·25	42·12	26·20	·9305	44·41	52·06	8·77
·9480	35·50	42·40	25·70	·9300	44·64	52·29	8·36
·9475	35·75	42·67	25·22	·9295	44·86	52·53	7·94
·9470	36·00	42·95	24·74	·9290	45·09	52·77	7·52
·9465	36·28	43·26	24·20	·9285	45·32	53·01	7·10
·9460	36·56	43·56	23·66	·9280	45·55	53·24	6·70
·9455	36·83	43·87	23·12	·9275	45·77	53·48	6·27
·9450	37·11	44·18	22·58	·9270	46·00	53·72	5·86
·9445	37·39	44·49	22·04	·9265	46·23	53·95	5·45
·9440	37·67	44·79	21·50	·9260	46·46	54·19	5·03
·9435	37·94	45·10	20·96	·9255	46·68	54·43	4·62
·9430	38·22	45·41	20·43	·9250	46·91	54·66	4·20
·9425	38·50	45·71	19·89	·9245	47·14	54·90	3·80
·9420	38·78	46·02	19·36	·9240	47·36	55·13	3·38
·9415	39·05	46·32	18·83	·9235	47·59	55·37	2·97
·9410	39·30	46·59	18·36	·9230	47·82	55·60	2·56
·9405	39·55	46·86	17·88	·9225	48·05	55·83	2·15
·9400	39·80	47·13	17·40	·9220	48·27	56·07	1·74
·9395	40·05	47·40	16·93	·9215	48·50	56·30	1·33
·9390	40·30	47·67	16·46	·9210	48·73	56·54	0·92
·9385	40·55	47·94	15·98	·9205	48·96	56·77	0·50
·9380	40·80	48·21	15·50	·9200	49·16	56·98	0·14
·9375	41·05	48·48	15·04	·9198	49·24	57·06	Proof

ALCOHOL TABLE.

Over Proof.

Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.	Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.
·9195	49·89	57·20	0·25	·8970	59·89	67·11	17·61
·9190	49·64	57·45	0·68	·8965	59·61	67·32	17·98
·9185	49·86	57·69	1·10	·8960	59·83	67·53	18·34
·9180	50·09	57·92	1·51	·8955	60·04	67·73	18·70
·9175	50·30	58·14	1·89	·8950	60·26	67·93	19·05
·9170	50·52	58·36	2·28	·8945	60·46	68·13	19·39
·9165	50·74	58·58	2·66	·8940	60·67	68·33	19·74
·9160	50·96	58·80	3·05	·8935	60·88	68·52	20·08
·9155	51·17	59·01	3·41	·8930	61·08	68·72	20·42
·9150	51·38	59·22	3·78	·8925	61·29	68·91	20·77
·9145	51·58	59·43	4·14	·8920	61·50	69·11	21·11
·9140	51·79	59·63	4·50	·8915	61·71	69·30	21·45
·9135	52·00	59·84	4·87	·8910	61·92	69·50	21·79
·9130	52·23	60·07	5·27	·8905	62·14	69·71	22·16
·9125	52·45	60·30	5·67	·8900	62·36	69·92	22·53
·9120	52·68	60·52	6·07	·8895	62·59	70·14	22·91
·9115	52·91	60·74	6·47	·8890	62·82	70·35	23·29
·9110	53·13	60·97	6·86	·8885	63·04	70·57	23·66
·9105	53·35	61·19	7·23	·8880	63·26	70·77	24·02
·9100	53·57	61·40	7·61	·8875	63·48	70·97	24·37
·9095	53·78	61·62	7·99	·8870	63·70	71·17	24·73
·9090	54·00	61·84	8·36	·8865	63·91	71·38	25·09
·9085	54·24	62·07	8·78	·8860	64·13	71·58	25·44
·9080	54·48	62·31	9·20	·8855	64·35	71·78	25·79
·9075	54·71	62·55	9·62	·8850	64·57	71·98	26·15
·9070	54·95	62·79	10·03	·8845	64·78	72·18	26·50
·9065	55·18	63·02	10·44	·8840	65·00	72·38	26·85
·9060	55·41	63·24	10·84	·8835	65·21	72·58	27·19
·9055	55·64	63·46	11·24	·8830	65·42	72·77	27·52
·9050	55·86	63·69	11·64	·8825	65·63	72·96	27·85
·9045	56·09	63·91	12·03	·8820	65·83	73·15	28·19
·9040	56·32	64·14	12·41	·8815	66·04	73·34	28·52
·9035	56·55	64·36	12·80	·8810	66·26	73·54	28·87
·9030	56·77	64·58	13·18	·8805	66·48	73·73	29·22
·9025	57·00	64·80	13·57	·8800	66·70	73·93	29·57
·9020	57·22	65·01	13·92	·8795	67·91	74·13	29·92
·9015	57·42	65·21	14·27	·8790	67·13	74·33	30·26
·9010	57·63	65·41	14·62	·8785	67·33	74·52	30·59
·9005	57·83	65·61	14·97	·8780	67·54	74·70	30·92
·9000	58·05	65·81	15·33	·8775	67·75	74·89	31·25
·8995	58·27	66·03	15·72	·8770	67·96	75·08	31·58
·8990	58·50	66·25	16·11	·8765	68·17	75·27	31·90
·8985	58·73	66·47	16·49	·8760	68·38	75·45	32·23
·8980	58·95	66·69	16·88	·8755	68·58	75·64	32·56
·8975	59·17	66·90	17·25	·8750	68·79	75·83	32·89

ALCOHOL TABLE.

Over Proof.

Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.	Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.
·8745	69·00	76·01	33·21	·8520	78·52	84·27	47·70
·8740	69·21	76·20	33·54	·8515	78·72	84·44	47·98
·8735	69·42	76·39	33·86	·8510	78·92	84·60	48·27
·8730	69·63	76·57	34·19	·8505	79·12	84·77	48·56
·8725	69·83	76·76	34·51	·8500	79·32	84·93	48·84
·8720	70·04	76·94	34·84	·8495	79·52	85·10	49·13
·8715	70·24	77·12	35·14	·8490	79·72	85·26	49·38
·8710	70·44	77·29	35·45	·8485	79·92	85·42	49·67
·8705	70·64	77·46	35·76	·8480	80·13	85·59	50·00
·8700	70·84	77·64	36·07	·8475	80·33	85·77	50·31
·8695	71·04	77·82	36·37	·8470	80·54	85·94	50·61
·8690	71·25	78·00	36·69	·8465	80·75	86·11	50·91
·8685	71·46	78·18	37·01	·8460	80·96	86·28	51·21
·8680	71·67	78·36	37·33	·8455	81·16	86·45	51·50
·8675	71·88	78·55	37·65	·8450	81·36	86·61	51·78
·8670	72·09	78·73	37·98	·8445	81·56	86·77	52·06
·8665	72·30	78·93	38·32	·8440	81·76	86·93	52·34
·8660	72·52	79·12	38·65	·8435	81·96	87·09	52·62
·8655	72·74	79·31	38·99	·8430	82·15	87·24	52·90
·8650	72·96	79·50	39·32	·8425	82·35	87·40	53·16
·8645	73·17	79·68	39·64	·8420	82·54	87·55	53·43
·8640	73·38	79·86	39·96	·8415	82·73	87·70	53·70
·8635	73·58	80·04	40·27	·8410	82·92	87·85	53·96
·8630	73·79	80·22	40·60	·8405	83·12	88·00	54·23
·8625	74·00	80·40	40·91	·8400	83·31	88·16	54·50
·8620	74·23	80·60	41·26	·8395	83·50	88·31	54·75
·8615	74·45	80·80	41·61	·8390	83·69	88·46	55·07
·8610	74·68	81·00	41·96	·8385	83·88	88·61	55·28
·8605	74·91	81·20	42·31	·8380	84·08	88·76	55·55
·8600	75·14	81·40	42·66	·8375	84·28	88·92	55·85
·8595	75·36	81·60	43·00	·8370	84·48	89·08	56·10
·8590	75·59	81·80	43·35	·8365	84·68	89·24	56·38
·8585	75·82	82·00	43·70	·8360	84·88	89·39	56·66
·8580	76·04	82·19	44·04	·8355	85·08	89·55	56·93
·8575	76·25	82·37	44·35	·8350	85·27	89·70	57·20
·8570	76·46	82·54	44·66	·8345	85·46	89·84	57·45
·8565	76·67	82·72	44·97	·8340	85·65	89·99	57·71
·8560	76·88	82·90	45·28	·8335	85·85	90·14	57·97
·8555	77·08	83·07	45·60	·8330	86·04	90·29	58·23
·8550	77·29	83·25	45·90	·8325	86·23	90·43	58·48
·8545	77·50	83·43	46·20	·8320	86·42	90·58	58·74
·8540	77·71	83·60	46·51	·8315	86·62	90·73	59·00
·8535	77·92	83·78	46·82	·8310	86·81	90·88	59·26
·8530	78·12	83·94	47·11	·8305	87·00	91·02	59·51
·8525	78·32	84·11	47·40	·8300	87·19	91·17	59·77

ALCOHOL TABLE.

Over Proof.

Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.	Sp. Gr. at 60° F.	Per cent of Alcohol by Weight.	Per cent of Alcohol by Volume.	Per cent over Proof.
·8295	87·38	91·31	60·02	·8110	94·28	96·32	68·80
·8290	87·58	91·46	60·28	·8105	94·45	96·43	69·00
·8285	87·77	91·60	60·53	·8100	94·62	96·55	69·20
·8280	87·96	91·75	60·79	·8095	94·80	96·67	69·40
·8275	88·16	91·90	61·05	·8090	94·97	96·78	69·61
·8270	88·36	92·05	61·32	·8085	95·14	96·90	69·82
·8265	88·56	92·21	61·60	·8080	95·32	97·02	70·03
·8260	88·76	92·36	61·86	·8075	95·50	97·15	70·25
·8255	88·96	92·51	62·12	·8070	95·68	97·27	70·46
·8250	89·16	92·66	62·38	·8065	95·86	97·39	70·67
·8245	89·35	92·80	62·63	·8060	96·03	97·51	70·88
·8240	89·54	92·94	62·88	·8055	96·20	97·62	71·07
·8235	89·73	93·09	63·13	·8050	96·37	97·73	71·26
·8230	89·92	93·23	63·38	·8045	96·53	97·83	71·45
·8225	90·11	93·36	63·62	·8040	96·70	97·94	71·64
·8220	90·29	93·49	63·84	·8035	96·87	98·05	71·83
·8215	90·46	93·62	64·06	·8030	97·03	98·16	72·02
·8210	90·64	93·75	64·30	·8025	97·20	98·27	72·20
·8205	90·82	93·87	64·51	·8020	97·37	98·37	72·40
·8200	91·00	94·00	64·74	·8015	97·53	98·48	72·58
·8195	91·18	94·13	64·96	·8010	97·70	98·59	72·77
·8190	91·36	94·26	65·18	·8005	97·87	98·69	72·95
·8185	91·54	94·38	65·40	·8000	98·03	98·80	73·14
·8180	91·71	94·51	65·62	·7995	98·19	98·89	73·30
·8175	91·89	94·64	65·85	·7990	98·34	98·98	73·47
·8170	92·07	94·76	66·07	·7985	98·50	99·07	73·64
·8165	92·26	94·90	66·30	·7980	98·66	99·16	73·81
·8160	92·44	95·03	66·53	·7975	98·81	99·26	73·97
·8155	92·63	95·16	66·76	·7970	98·97	99·35	74·14
·8150	92·81	95·29	67·00	·7965	99·13	99·45	74·31
·8145	93·00	95·42	67·23	·7960	99·29	99·55	74·50
·8140	93·18	95·55	67·46	·7955	99·45	99·65	74·66
·8135	93·37	95·69	67·70	·7950	99·61	99·75	74·83
·8130	93·55	95·82	67·92	·7945	99·78	99·86	75·01
·8125	93·74	95·95	68·15	·7940	99·94	99·96	75·18
·8120	93·92	96·08	68·38	·7938	Absolute	Alcohol	75·25
·8115	94·10	96·20	68·60				

(2) Berthelot's benzoyl-chloride test. The liquid is shaken with a few drops of benzoyl-chloride. Alcohol reacts with this, forming ethyl benzoate which, if present, sinks to the bottom with excess of benzoyl-chloride. The heavy layer is drawn off and warmed with a little caustic potash solution. The characteristic odour of ethyl benzoate is at once perceived.

(3) Hardy's test. This test is carried out as follows: two ordinary Nessler cylinders are stood side by side on a white tile. Into one is placed the sample to be tested, after being well shaken up with a fragment of guaiacum resin which has been freshly detached from a lump, and then filtered. A few drops of dilute hydrocyanic acid and a drop or two of dilute copper sulphate solution are then added. The same procedure is adopted with the second tube, except that distilled water is used in place of the sample. If alcohol be present, the sample will have a much deeper blue colour than the blank cylinder.

Water in Alcohol.—In the examination of absolute alcohol, the presence of water is important; 0.4 per cent of water may be detected by shaking the liquid with a crystal of potassium permanganate. In the presence of this amount of water, the liquid will acquire a pink tinge, whereas potassium permanganate is totally insoluble in alcohol. If alcohol be shaken with anhydrous cupric sulphate it will give the salt a blue colour in the presence of about 0.8 per cent of water, or calcium carbide may be shaken with the alcohol. In the absence of water no reaction takes place, whilst in the presence of water, bubbles of acetylene are liberated and the liquid becomes turbid owing to the formation of traces of calcium hydroxide.

The Detection of Methyl Alcohol.—The following methods may be used:—

(1) Mulliken and Scudder's test depends on the oxidation of methyl alcohol to formic aldehyde by means of a hot copper wire. It has been modified and adopted by the United States Pharmacopœia and will, in this form, detect as little as 2 per cent of methyl alcohol in ethyl alcohol. It is carried out as follows:—

Into a test tube 1 c.c. of the preparation to be tested is introduced, and, unless dilute, made up to 10 c.c. The proportion of alcohol present should not exceed 10 per cent by volume. A copper wire spiral is made by winding 1 metre of No. 18 copper wire closely round a glass rod 7 mm. thick, making a coil 3 cm. long, the rest of the wire being used for a handle. The coil is heated to redness in a smokeless flame, then immersed steadily quite to the bottom of the alcoholic fluid. This treatment is repeated five or six times, immersing the tube meanwhile in cold water to keep down the temperature of the liquid. The spirit is now filtered into a wide test tube and boiled very gently. If any odour of acetaldehyde is perceptible, boiling is continued until this has been dissipated. The liquid is then cooled and a drop of 1 : 200 solution of resorcinol added to it. A portion of this mixture is then floated on H_2SO_4 in another tube and allowed to stand for three minutes, then slowly rotated. No rose-red ring should be evident at the zone of contact of the two liquids, indicating the absence of more than 2 per cent of methyl alcohol.

(2) Riche and Bardy's method. Ten c.c. of the sample, rectified over potassium carbonate if necessary, are placed in a flask with 15 grms. of iodine and 2 grms. of red phosphorus. The flask is kept in ice-cold water until the reaction is over. The ethyl and methyl iodides are then distilled into about 30 c.c. of water. The free iodine may be removed by washing with dilute alkali. The heavy oil is now

separated and transferred to a flask containing 5 c.c. of aniline. The flask should be kept in cold water in case the reaction is violent, or it may be warmed if the reaction appears to require stimulating. After an hour the reaction product is boiled with water, and about 20 c.c. of a 15 per cent solution of caustic soda added. The bases formed now rise to the surface of the fluid and are separated. To 1 c.c. of the oil add 10 grms. of a mixture of 100 parts of sand, 2 of common salt, and 3 of cupric nitrate. After thorough mixing, introduce into a glass tube and heat to 90° C. for eight to ten hours. The product is then exhausted with warm alcohol, the alcohol filtered and made up to 100 c.c. If the sample were free from methyl alcohol, the liquid is of a red tint, but in the presence of 1 per cent of methyl alcohol, it has a distinct violet shade. With 2.5 per cent the violet is very marked. Even smaller quantities are indicated by diluting 5 c.c. of the coloured alcoholic extract to 100 c.c. with water, and again diluting 5 c.c. of this to 400 c.c. If this be now heated and a fragment of white wool, absolutely free from sulphur, be inserted in it for an hour, it will be dyed a faint violet, if traces of methyl alcohol are present.

(3) Trillat's test. This test is a very delicate but very tedious one. To 50 c.c. add 8 grms. of lime, and 50 c.c. of water, and then distil with the aid of a pear head, or Glinsky bulb tubes. Dilute the first 15 c.c. distilled to 150 c.c. with water, and add 15 grms. of potassium bichromate and 70 c.c. of 20 per cent sulphuric acid. Allow the whole to stand for an hour with occasional shaking. Redistil, rejecting the first 25 c.c. and reserving 100 c.c.; 50 c.c. of this are mixed with 1 c.c. of pure dimethyl-aniline and transferred to a well-stoppered, stout glass flask. The mixture is rendered distinctly alkaline with caustic soda, and the excess of dimethyl-aniline distilled off, the distillation being stopped when 25 c.c. have come over. The residue in the flask is acidified with acetic acid, well shaken and 5 c.c. of water containing 1 per cent of lead dioxide in suspension added. If methyl alcohol be present, a blue coloration is developed which is increased by boiling (ethyl alcohol yields a blue colour, changing at once to green and then to yellow, becoming colourless when boiled).

(4) Hinkel's method. This is not particularly delicate, only detecting about 4 to 5 per cent of methyl alcohol in ethyl alcohol. But since in preparations such as tinctures, etc., when it is likely methylated spirit might be used, the amount of methyl alcohol is usually appreciable, if any be present at all, the method is frequently of service.

To 1 c.c. of the alcoholic distillate 0.8 gm. of ammonium persulphate and 3 c.c. of 20 per cent sulphuric acid are added. The whole is diluted with water to 20 c.c. and distilled. The distillate is collected in fractions of 2 c.c. of which the first five are reserved. The first two, containing acetaldehyde, are rejected, the remaining three being tested as follows:—

A few drops of a 0.5 per cent solution of morphine hydrochloride are added to each, and then strong H_2SO_4 is added to form a layer at the bottom of the liquid. In the presence of formic aldehyde, which

indicates the presence of methyl alcohol, a violet ring is formed at the junction of the liquid. As this reaction will detect minute quantities of formic aldehyde, and as pure alcohol yields traces of formic aldehyde, a control experiment should be conducted on pure ethyl alcohol, so that the colours may be compared.

Where very small quantities of methyl alcohol are in question the alcohol should be fractionally distilled under a pear head or rod and disc apparatus, and the earlier fraction subjected to one or more of the above tests.

Sanglé-Ferrière and Cuniasse detect methyl alcohol as follows. ("Annales de Chim. Analyt." **8**, 82): 50 c.c. of the liquid is distilled off, the distillate acidulated with 1 c.c. of pure H_2SO_4 , and treated with 5 c.c. of saturated solution of $\text{K}_2\text{Mn}_2\text{O}_8$. After allowing to stand for a few minutes the colour should be distinctly brown, without any reddish tinge due to excess of $\text{K}_2\text{Mn}_2\text{O}_8$. If this excess should occur it must be removed by the addition of a drop or two of solution of tannin. The liquid is then made faintly alkaline with Na_2CO_3 , filtered, and treated with 2 c.c. of a 1 per mille solution of phloroglucin and 1 c.c. of strong solution of KOH . In the presence of added methyl alcohol a marked red colour reaction will be obtained. A slight yellowish-red or violet tint may be disregarded, since a trace of methyl alcohol may occur in pure wine alcohol: the reaction, due to added methyl alcohol, being bright red, is unmistakable. A confirmatory test may be obtained with gallic acid. The alkaline filtrate is acidified with a dilute H_2SO_4 ; a few grains of gallic acid are dissolved in the liquid, when a few drops of strong H_2SO_4 are carefully run down to the bottom of the vessel. In the presence of methyl alcohol a blue colour will form at the zone of contact of the two liquids. It will be seen that these reactions depend on the formation of formaldehyde by the oxidation of the methyl alcohol.

The Determination of Methyl Alcohol.—The method of Thorpe and Holmes ("J. Chem. Soc." 1904, **85**, 1) is accurate and comparatively simple. The sample is mixed with water so that 50 c.c. shall contain not more than 1 gm. of methyl alcohol, or 4 grms. of mixed methyl and ethyl alcohols. Fifty c.c. of this mixture are placed in a 300 c.c. flask, which can be closed by a ground-in stopper, and which is fitted with a funnel and side tube; 20 grms. of potassium bichromate are added and 80 c.c. of 25 per cent H_2SO_4 . The mixture is allowed to stand for eighteen hours. A further quantity of 10 grms. of potassium bichromate and 100 c.c. of 50 per cent H_2SO_4 (by volume) are now added, and the liquid raised to the boiling-point for ten minutes, the CO_2 evolved being swept out of the flask by a current of air, and after passing through drying tubes of CaCl_2 and H_2SO_4 , collected in a soda lime tube and weighed. Thirty-two parts of methyl alcohol yield forty-four parts of CO_2 . A correction must be made for the ethyl alcohol present by deducting 0.01 gm. of CO_2 for each gm. of ethyl alcohol. As there is always at least ten times as much ethyl alcohol present as methyl alcohol, the amount of ethyl alcohol may be taken as that indicated by the specific gravity of the liquid or distillate. Certain other constituents of wood naphtha are

completely oxidized to CO_2 in this manner, so that the above results are comparative rather than absolute, being from 5 to 10 per cent above the actual truth.

Thorpe and Holmes recommend, in the case of tinctures, that the alcohol from 25 to 50 c.c. of the sample be distilled, and any essential oils present removed by shaking with petroleum ether, and then distilled and diluted to 250 c.c. Fifty c.c. of this mixture are then treated as above described. If the weight of CO_2 does not exceed 0.01 gm. per gm. of alcohol present, it may be concluded that methyl alcohol is absent.

An alternative method is that devised by Leach and Lythgoe, which depends on the fact that the refractive indices of methyl and ethyl alcohols are very different. These chemists use the Zeiss immersion refractometer, but any form of refractometer will be found suitable so long as the absolute index of refraction be determined. As most chemists use a Zeiss Abbé refractometer, the following values refer to this treatment. It is only necessary to distil say 75 per cent of the liquid and make up to original volume and determine the specific gravity. The refractive index at 20° is then taken. If the amount of alcohol as indicated by the specific gravity agrees with that indicated by the refractive index, no methyl alcohol is present. If a difference is indicated, the amounts of methyl alcohol present can be deduced by interpolation and calculation. The table on page 284 gives the amounts of the two alcohols indicated by various refractive indices:—

For example, if the distillate showed a specific gravity which corresponded, as found by the tables, to 18 per cent of ethyl alcohol, and has a refractive index 1.34116.

The correct readings for ethyl and methyl alcohols of 18 per cent strength are 1.34518 and 1.33712 respectively, the difference being 0.00806. The difference between the refractive index of pure ethyl alcohol of this strength and of the sample is $1.34518 - 1.34116 = 0.00402$.

So that $0.00402 : 0.00806 :: x : 100$, where x is the amount of methyl alcohol in the total alcohol present, that is, in the above case, 49.9 per cent.

The Determination of Alcohol.—Where only alcohol and water are present, it is sufficient to determine the specific gravity, from which the amount of alcohol is at once found from the table on p. 275. In the presence of fixed matter (volatile acids may be fixed by neutralization with alkali) 100 c.c. (if possible) should be distilled until 80 c.c. has been collected, and this should then be made up to the original volume with distilled water. In the case of many wines the addition of a little tannin will be found to assist a quiet distillation. When the result has to be expressed by weight, the amount, by weight, in the distillate so made up to original volume is taken from the table and then corrected by multiplying by the factor $\frac{\text{sp. gr. of distillate}}{\text{sp. gr. of sample}}$, which then gives the amount by weight in the original sample.

An alternative method where fixed matter is present is to evapor-

ate a measured portion of the sample to about 25 per cent of its volume and when cold make up to its original volume with distilled water

Per cent by Weight of Alcohol.	Refractive Indices.	
	Methyl Alcohol.	Ethyl Alcohol.
	Per cent	Per cent
1	1·33312	1·33358
2	1·33335	1·33420
3	1·33358	1·33478
4	1·33381	1·33540
5	1·33405	1·33601
6	1·33427	1·33671
7	1·33451	1·33739
8	1·33474	1·33812
9	1·33497	1·33881
10	1·33521	1·33949
11	1·33543	1·34018
12	1·33567	1·34086
13	1·33590	1·34158
14	1·33613	1·34226
15	1·33636	1·34294
16	1·33663	1·34369
17	1·33686	1·34444
18	1·33712	1·34518
19	1·33735	1·34593
20	1·33762	1·34668
22	1·33812	1·34809
24	1·33862	1·34954
26	1·33907	1·35091
28	1·33957	1·35223
30	1·34002	1·35352
32	1·34052	1·35450
34	1·34094	1·35547
36	1·34135	1·35638
38	1·34173	1·35718
40	1·34203	1·35797
42	1·34229	1·35869
44	1·34248	1·35937
46	1·34256	1·36002
48	1·34264	1·36063
50	1·34267	1·36120
52	1·34260	1·36149
54	1·34256	1·36217
56	1·34245	1·36255
58	1·34222	1·36294
60	1·34195	1·36329
62	1·34162	1·36362
64	1·34124	1·36394
66	1·34086	1·36419
68	1·34048	1·36443
70	1·34010	1·36464

and take the specific gravity of this de-alcoholized liquid. Add 1·000 to the original specific gravity and subtract the second gravity. The difference is the specific gravity corresponding to the alcohol present,

from which the amount of alcohol is determined by reference to the table. Thus, if the specific gravity of the original sample be 0.9850, and that of the de-alcoholized sample is 1.0040. Then $1.9850 - 1.0040 = 0.981$, and this corresponds to 15.1 per cent by volume of alcohol. To convert this into the percentage by weight it should be multiplied by 0.7938 and divided by the specific gravity of the original liquid.

The determination of alcohol by the vaporimeter is described under wines (p. 315). Wiley ("Journ. Amer. Chem. Soc." 1896, **18**, 1063) has described a method for the determination of alcohol based on the boiling-point of the liquid. For the details of this method the original paper should be consulted, but the following remarks may be made upon it. Where the alcoholic liquid contains solid matter in solution, the actual boiling-points of liquids are considerably altered, and a diluted whisky and a port wine, each containing the same quantity of alcohol, will give different boiling-point results. Where there is no solid matter present, the specific gravity gives accurate results, and so cumbersome a method as this is unnecessary.

Where the volatile substances are present, such as essential oils and the like, Thorpe and Holmes ("Journ. Chem. Soc." 1903, **83**, 314) use the following process: 25 c.c. of the sample, at 60° F., are mixed with water in a separator to a volume of 100 to 150 c.c. and sodium chloride added in sufficient quantity to saturate the liquid. The mixture is now shaken well with petroleum ether (50 to 80 c.c.), and after standing for half an hour the lower layer is drawn off and extracted again with petroleum ether and then drawn off again, and the petroleum ether liquid washed twice with salt solution, and the washings added to the main bulk of the liquid and the whole distilled, and the distillate made up to 100 c.c. (four times the original bulk). From the specific gravity of the distillate the amount of alcohol is at once found. If ammonia be present, the liquid must be rendered slightly acid. If camphor be present, dilute sulphuric acid is better to use than salt.

Determination of Higher Alcohols in Spirits of Wine.—C. Bardy ("Comptes Rendus," cxix. 1201-1204) recommends the following process for the determination of higher alcohols in spirits of wine: A preliminary examination is made by agitating 10 c.c. of the alcohol to be tested with 50 c.c. of saturated solution of sodium chloride. Two results may thus be produced:—

1. The salt solution forms a clear mixture with the alcohol, thus indicating that the amount of impurity is small. In this case 500 c.c. of the alcohol are mixed in a capacious separator with 450 c.c. of solution of sodium chloride, and subsequently with sufficient water to re-dissolve the salt separated; 60 c.c. to 70 c.c. of carbon bisulphide are then added, the whole is well shaken, and after some minutes' rest the bisulphide is separated. This operation is repeated three times. The bisulphide will then contain the whole of the butyl and amyl alcohols, and to extract these it is shaken with 2 c.c. of strong sulphuric acid, and the acid removed, after settling, into a flask of 125 c.c. capacity. This operation is also repeated several times, and the united acid liquor is freed from bisulphide by warming. An

equal volume of glacial acetic acid is now added, the neck of the flask closed with a reflux condenser, and the mixture heated to 100° C. for four hours to promote formation of acetic ethers. The contents of the flask are then mixed with 100 c.c. of salt solution; if higher alcohols were present, the ethers will separate as an oily layer on the surface. This oily liquid is separated and measured at 15° C.; the volume expressed in c.c. and multiplied by 0.8 gives the percentage of butyl and amyl alcohols.

2. An oily layer separates at the surface of the salt solution in the preliminary experiment. In that case larger amounts of the higher alcohols are present, and the operations above described are now carried out with a smaller quantity (25 c.c.) of the alcohol, 100 c.c. of saturated salt solution, and 8 to 10 c.c. of water. The quantity of bisulphide of carbon should not be reduced. Since the latter dissolves only the butyl and amyl alcohols, the liquid from which the bisulphide has been separated must be examined for propyl and isopropyl alcohols. For this purpose it is filtered through moist paper and distilled, the distillate being collected in a tube containing an alcoholometer until this instrument indicates 50°. At that point the whole of the propyl alcohol will have passed over, and may be determined in the distillate by titration with permanganate.

BRANDY.

Brandy is a spirit resulting from the distillation of fermented grape juice or wine. Apart from the question of alcoholic strength, for which a *prima facie* standard exists, the only question with which the analyst is usually concerned is the admixture with true brandy of alcohol derived from other sources, a form of adulteration which is referred to in the oldest books available. Brandy is a term sometimes applied to a spirit derived from other sources than the grape, but if the use of the word is at all justifiable in this sense it should certainly be qualified in such a manner that the source is indicated, e.g. plum brandy. The following definition of brandy has been agreed to by the recent Royal Commission on whisky and other potable spirits, 1909. "The term 'brandy' is applicable to a potable spirit manufactured from fermented grape juice, and from no other materials."

They are, however, of opinion that the compounded spirit long recognized by the name of British brandy is entitled still to be so named and sold as "British brandy".

The bulk of the brandy of commerce is prepared in France, but, of course, pure brandy is made in other countries. The most esteemed type of spirit is that distilled in the Charente district, and it is reasonable that the term Cognac should, in this country, carry the same meaning as that which it does officially in the home of the industry. By a decree dated 1 May, 1909, of the French Republic, no brandy shall be entitled to the name "Cognac," "Eau-de-vie de Cognac" or "eau de vie des Charente," except it be distilled on the spot from vines grown in the following districts:—

1. *Department of Charente-Inferieure*.—The arrondissements of Rochefort, Marennnes, Saintes, St. Jean d'Angely, Jonzac, parts of La Rochelle.

2. *Department of Charente*.—The arrondissements of Cognac, Barbezieux, parts of Angoulême, parts of Ruffec.

3. *Department of Dordogne*.—Parts of the arrondissement of Ribérac.

4. *Department of Deux Sevres*.—Parts of the arrondissements of Niort and Melle.

The French Government have further restricted the use of the names Armagnac and Ténarèze to brandies distilled from wine grown and made within suitable geographical limits (Decree of 25 May, 1909).

The functions of the analyst are principally of importance, however, in deciding whether a brandy is pure, and no chemical means are available for discriminating between pure brandies. The trade expert would, however, be able to decide the place of origin of a brandy with a fair degree of accuracy.

There can be no reasonable doubt that the medicinal value of brandy is not entirely due to the alcohol it contains, but also to the secondary constituents, which are either the result of the original fermentation and distillation, or are formed during the process of maturing. The characteristic flavour of brandy is, in the same manner, due to such secondary constituents. Vasey ("Potable Spirits") has remarked that "the patent or fractionating still is practically the key to the situation as regards the analysis of potable spirits". This is, of course, true of brandy, whisky, and rum at all events, and requires comment at the present stage.

It is not proposed to enter into controversial matter as to the actual merits of the pot or simple still, as against the patent or fractionating still. Ample details of this—an essentially trade matter—will be found in the report of the recent Royal Commission on whisky and other potable spirits. The facts, however, amount to the following, which apply equally to all such distilled spirits, but principally to whisky:—

1. The distillate from the pot still contains considerably more secondary constituents, as would be expected, than does the distillate from a patent still.

2. The presence of a large amount of these secondary constituents renders it necessary to mature a pot-still product for a considerable time, in order to allow sufficient change in the character of the secondary constituents to take place for the spirit to be palatable. As to the physiological effect of new and matured pot-still spirits, great differences of opinion exist.

3. Patent-still spirits contain very little secondary constituents and thus require but little maturing, but are usually correspondingly flavourless. They approximate in character to a pure diluted alcohol obtained from any source, more or less, according to the nature of the still.

In regard to whisky, as will be seen later, it appears that no attempts will be made to restrict the patent stills which have been

in use for very many years, as the beverage sold as whisky, made from grain spirit, has for many years been either a pot-still product or a blend of the two distillates. It is therefore a matter of the public taste—"de gustibus non est disputandum".

The brandy question is, however, not quite on the same footing, and from the point of view of public expediency it is desirable that stills of the pot or simple type should be used for the manufacture of brandy.

The addition of spirit from other sources than the grape to brandy is, of course, deliberate adulteration.

Incidentally it may be mentioned that the British Pharmacopœia defines brandy as a spirituous liquid distilled from wine and matured by age, and containing not less than 36·5 per cent by weight or 43·5 per cent by volume of alcohol.

Freshly distilled brandy is colourless, the colour of commercial brandy being due to colouring matter derived from the casks in which it is stored.

Brandy may be sold as such, diluted with water so that the strength is not below 25° under proof (= 35·93 per cent by weight, or 42·8 per cent by volume). The secondary constituents of brandy have been examined with minute care by Ordonneau ("Comptes Rendus," *CH.* 217). The typical sample upon which his results were based was a Cognac brandy twenty-five years old.

He found in each 100 litres of absolute alcohol present :—

	Grms.
Acetic aldehyde	3
Acetal	35
Butyl alcohol	218·6
Hexyl "	1·5
Propionic, butyric, and caproic esters	3
Amine bases	4
Ethyl acetate	35
Propyl alcohol	40
Amyl "	83·6
Oenanthic ether	4

It appears to be agreed that the principal flavouring constituent of brandy is oenanthic ether, and as this is easily made artificially from the products of distillation of castor oil, it forms the basis of so-called "artificial cognac oil," which is sold in order to mix with "silent" spirit in order to prepare a factitious brandy.

The higher alcohols in brandy, separated by Ordonneau and by Claudon and Morin under the name "fusel oil," have been carefully examined, and the results of both investigations are very concordant. According to these authorities the composition is as follows :—

	Ordonneau.	Claudon and Morin.
	Per cent	Per cent
Propyl alcohol	11·9	11·7
Normal butyl alcohol	49·3	63·8
Isobutyl alcohol	4·5	0·0
Amyl alcohol	34·4	24·5

The higher alcohols of potato spirit have approximately the following composition :—

	Per cent
Isopropyl alcohol	15
Isoamyl „	30
Butyl „	8
Isobutyl „	5
Amyl „	18
Other bodies	26

It is impossible to estimate individual compounds in a spirit, but allied substances can be determined in groups, which materially assist the analyst in coming to a conclusion.

But one cannot emphasize too strongly that any standards that have been published for limits of esters, aldehydes, etc., are liable to be found untrue for occasional cases. The author is acquainted with some of the finest champagne brandies which invariably show a deficiency in esters according to the standards generally accepted, whereas by “doctoring” with a trace of artificial cognac oil, they will at once appear to pass the standards.

In this regard, attention should be paid to the remarks contained in the final report of the Royal Commission on whisky and other potable spirits, 1909. The Commissioners state that whilst certain benefits have been obtained from the adoption of an “ether standard” of 80 parts of ethers per 100,000 parts of absolute alcohol (a standard largely adopted by public analysts), the adoption of such a standard based on a minimum quantity of ethers alone is quite incapable of affording general protection against fraud. For instance, genuine brandies are frequently found to contain upwards of 100 parts of ethers per 100,000 of absolute alcohol: admixtures of such with 20 per cent of neutral spirit would be passed as genuine brandies by the standard. Again, the requisite proportion of ethers in any spirit can be insured by the addition of suitable ethers. The standard can therefore no longer be regarded as useful, and may even be mischievous. All the usual analytical data, and in addition, the flavour, and any information obtainable from the excise records as to the origin of the sample should be taken into account in order to form a reliable opinion as to the genuineness or otherwise of a sample of reputed brandy.

There is no doubt that this is true, but it must be remembered that there is one case, and a not uncommon one, where the ether value, as the Commissioners term it, is of definite service, that is, where it falls *materially* below 80. For example, spirit with an ether value of 25 to 50, as often met with, would be condemned as containing neutral spirit on all hands.

The quantitative determinations hereinafter described, are therefore to be judged in the light of the above remarks.

Genuine brandy contains a small amount of volatile acids of the acetic acid series; aldehydes of the aliphatic series, and traces of furfural; esters and higher alcohols.

The examination of brandy should include the determination of

alcoholic strength; solid residue; free volatile acids calculated as acetic acid; total aldehydes; furfural; esters calculated as ethyl acetate, and higher alcohols.

Alcohol.—When the solid residue is under 0·5 per cent, as it frequently is, the specific gravity of the sample is a sufficiently correct indication of the amount of alcohol present. If a high residue be present, 50 c.c. should be distilled from 60 c.c. and the distillate made up to 60 c.c., and the specific gravity taken.

Solid Residue.—The solid residue of genuine brandy averages under 1 per cent, usually from 0·3 per cent to 0·6 per cent, but sometimes, especially in brandies which have been stored in new casks, up to 3 per cent of solid residue may be present. It is customary to return the secondary constituents of brandy in terms of parts per 100,000 of absolute alcohol present. Thus if a brandy contain 40 per cent of alcohol by weight, and two parts of furfural per 100,000 be found, this would be returned as 5 per 100,000. The following represent the analyses of a large number of pure French brandies made by the author and various observers, whose names are given:—

Parts per 100,000 of Absolute Alcohol.						
No. of Samples.	Volatile Acids as Acetic.	Total Aldehydes.	Furfural.	Esters as Ethyl Acetate.	Higher Alcohols.	Observers.
	Per cent	Per cent	Per cent			
30 (average of)	81·5	21·5	1·6	116	138	Parry
New brandy	74·0	14·5	2·6	108	195	"
Brandy 25 years old. . .	119·5	27·8	1·15	126	219	"
" 40 " " "	202	48	1·20	133	345	Girard
" 6 " " " "	229	11·5	1·20	101	260	"
Various samples (average)	81·9	24·2	0·16	212	289	König
" " " " " " " "	120	21·0	1·34	87	165	Möhler

The following are types of spirit used in mixing with natural potable spirits:—

Parts per 100,000 of Absolute Alcohol.						
Samples.	Volatile Acids as Acetic.	Aldehydes.	Furfural.	Esters.	Higher Alcohols.	Observers.
	Per cent	Per cent	Per cent	Per cent	Per cent	
Grain spirit	2·5	0·1	nil	3·6	2·9	Girard
" " " " " " " "	8·4	4·9	0·35	23·8	traces	Vasey
" " " " " " " "	61·2	11·22	0·23	32·65	85	"
" " " " " " " "	nil	3·70	nil	47·6	67·7	Schidrowitz
Beetroot spirit	2·5	nil	nil	3·6	2·5	Girard
Potato " " " " " "	1·8	4·9	0·1	5·0	19·9	Parry
(average of 8 samples)						

The use of pure alcohol will be frequent in the examination of potable spirits, and it is essential that it shall contain none of the impurities which are estimated. Pure 90 per cent alcohol can easily be obtained free from esters and aldehydes, but should never be relied on unless carefully checked. To ensure a pure alcohol, it should first be boiled for an hour under a reflux condenser to destroy esters, and then, after distillation, be again boiled for an hour with 0.4 per cent of sodium phenyl-hydrazine parasulphonate or metaphenylamine diamine hydrochloride.

The Determination of Alcohol.—When exact determination is necessary 90 per cent of the liquid may be distilled and the distillate made up to the original volume and the specific gravity taken.

Approximately accurate results may be obtained by taking the specific gravity of the liquid (S), then evaporating off all the alcohol and making up to the original volume with water, and taking the specific gravity of this liquid (S'). Then $\frac{S}{S'}$ = specific gravity due to the diluted alcohol, from which the amount of alcohol is deduced.

The Free Acids.—Twenty-five c.c. of the sample are titrated with $\frac{N}{10}$ baryta water using phenol-phthalein as indicator. This gives the total acidity, which is expressed in terms of acetic acid. A second 25 c.c. are evaporated to dryness, the residue being redissolved in water and dried again twice, and finally again dissolved in water and titrated with $\frac{N}{10}$ baryta water. Each c.c. of $\frac{N}{10}$ baryta water corresponds to 0.0075 grm. of tartaric acid; the difference in the number of c.c. required for the fixed and total acidity $\times 0.006$ gives the amount in grams, of volatile acids calculated as acetic acid.

[The French official method, fixed by ministerial decree in 1907, requires the use of 25 c.c. for the total acidity, using $\frac{N}{20}$ soda solution. For the fixed acidity, the liquid is evaporated from 25 to 5 c.c., and the drying completed in vacuo.]

Total Aldehydes.—The best process for the determination of aldehydes which will include the furfural present, is the official process of the French Government, which has taken account of all the errors probable in similar previously described processes. It depends upon a comparison of the colours developed by the reaction of aldehyde and fuchsine-sulphurous acid. The precautions which must be taken are that the determinations should be carried on under such conditions that the standard and the sample are of as nearly as possible the same aldehydic strength, and the *alcoholic* strengths of the liquids must be practically identical. If colorimeters are used where the same tint is obtained on different thicknesses of the coloured solution, account must be taken of the fact that the colour developed is not in direct proportion to the amount of aldehyde present, and corrections must be made. In the following process, although approximate results may be obtained by any suitable colorimetric comparison, greater accuracy is ensured

by using a standard colorimeter such as that of Dubosq or Mills, and comparing the colour against 10 millimetres of the standard solution. If the thickness of the sample under examination be materially different from 10, the following table, due to Cuniasse, enables the necessary corrections to be made :—

Indication of colorimeter matching 10 mm. of standard described below.	Aldehydes per 100,000 of absolute alcohol.
100	4
40	9
25	12
16·7	15
10	20
6·9	25
5·4	30
4·2	35
3·4	40

The process is carried out as follows :—

A standard solution of 0·1 grm. of aldehyde per litre (10 per 100,000 c.c.) is prepared by washing commercial aldehyde-ammonia (which is stable) several times with ether, pouring off the ether and drying the crystals, which have been rubbed down in a mortar, over H_2SO_4 in vacuo. 1·386 grms. (= 1 grm. aldehyde) are dissolved in about 50 c.c. of 95 per cent alcohol. 22·7 c.c. of normal H_2SO_4 are added, when ammonia sulphate is precipitated. The liquid is made up to 100 c.c. with 95 per cent alcohol, and then 0·8 c.c. of alcohol is added to compensate for the volume of the sulphate of ammonia. The liquid is well shaken, left for twelve hours, and filtered. It is now a solution of 1 grm. of pure aldehyde in 100 c.c. of alcohol. About 90 c.c. of water are then added and the whole made up to 1000 c.c. with 50 per cent alcohol. It is now a 0·01 per cent solution of aldehyde in practically 50 per cent alcohol.

The fuchsine-sulphurous acid solution is made by mixing 30 c.c. of 0·1 per cent solution of fuchsine in 95 per cent alcohol, 15 c.c. of a solute of sodium bisulphite of specific gravity 1·308, and 30 c.c. of water. The mixture is shaken, and allowed to stand for an hour, and then 15 c.c. of 30 per cent sulphuric acid added. The liquid is then made up to 250 c.c. with 50 per cent alcohol. After standing for a short time this solution becomes quite colourless.

A portion of the 90 per cent distilled for the determination of the alcohol is used for the determination, which, however, is diluted with pure alcohol (so as to be of 50 per cent strength), or by water if above that strength.

In the comparisons 10 c.c. of the standard aldehyde solution are placed in one tube, and 10 c.c. of the diluted distillate in another. For the original alcoholic strength, the amount of dilution is calculated and the observed results corrected accordingly. For example, if 100 c.c. of the distillate have to be diluted to 130 c.c. for the determination, the observed results used have to be multiplied by 1·3.

Four c.c. of the fuchsine-sulphurous acid solution is added to each

tube and the contents well mixed and allowed to stand for twenty minutes when the reading can be taken.

For example: If from 100 c.c. of brandy 90 c.c. be distilled and made up to 100 c.c. and it is found that it is of 33 per cent strength, it is necessary to add sufficient 95 per cent alcohol to 100 c.c. to bring the volume up to 134.8 c.c. so as to bring the alcoholic strength to 50 per cent. Therefore the observed result must be multiplied by 1.348. If 10 c.c. of the so diluted distillate exactly match 10 c.c. of the standard, then it will contain 0.1 gm. of aldehyde per litre, but being of 50 per strength, this is 0.2 gm. per litre of absolute alcohol or 20 per 100,000. This multiplied by 1.348 is 26.96. The brandy therefore contains 26.96 parts of aldehyde per 100,000 parts of absolute alcohol. The tables on page 294 give the amounts of 95 per cent alcohol or water to be added to alcohols (distillates) in order to bring them to exactly 50 per cent strength by volume.

An alternative method for the estimation of the aldehydes is as follows, provided the amount of aldehydes is high, as is the case sometimes:—

The following solutions are necessary:—

(1) *Pure* sulphite of sodium (anhydrous) 12.6 grms. dissolved in 400 c.c., 100 grms. of normal sulphuric acid added, and the whole made up to 1000 c.c. with 95 per cent alcohol. If crystals of Na_2SO_4 separate they should be filtered off.

(2) Decinormal solution of iodine in iodide of potassium 1 c.c. = 0.0032 gr. SO_2 or 0.0022 of ethyl aldehyde.

Into a 100 c.c. flask, 10 c.c. of the solution to be tested (if the aldehydes are present to the extent of 0.5 per cent to 1 per cent, or correspondingly more, if the aldehyde value is lower) are placed, and 50 c.c. of the above sulphurous acid solution added. The volume is made up to 100 c.c. with 50 per cent alcohol. The whole is well shaken and the flask securely stoppered.

A blank experiment is conducted in the same manner, only omitting the solution to be tested. The two flasks, securely stoppered are placed in a water bath at 50° for four hours, and then cooled and well shaken. Fifty c.c. are then titrated with the iodine solution. The difference in the amount of free SO_2 indicates the amount that has continued with the aldehydes, each c.c. of decinormal iodine being equivalent to 0.0022 gm. of ethyl aldehyde.

Estimation of Furfural.—A standard solution of 10 milligrams of furfural per 1000 c.c., in 50 per cent alcohol is used.

Ten c.c. of the alcohol (distillate) brought to 50 c.c. strength, and 10 c.c. of the standard solution are each treated with 0.5 c.c. of freshly prepared aniline and 2 c.c. of glacial acetic acid. After twenty minutes the solutions are compared colorimetrically. The same remarks as to correction for dilution, and the irregular ratio of the colour produced to the amount of furfural, apply here as to the fuchsine-sulphurous acid method of determining aldehydes (p. 292). The following table will enable the observer to make the necessary corrections, but the greatest accuracy is obtained by repeating the experiment if the columns are much different with quantities so adjusted to give as nearly

Original Strength of Alcohol.	Volume of 95 per cent Alcohol to add to 100 Vols.	Final Volume Obtained.
Per cent	Per cent	Per cent
30	42.2	140.2
31	40.1	138.2
32	38.0	136.3
33	36.0	134.3
34	33.9	132.4
35	31.8	130.4
36	29.7	128.4
37	27.6	126.5
38	25.5	124.5
39	23.4	122.5
40	21.3	120.5
41	19.2	118.5
42	17.1	116.4
43	14.9	114.4
44	12.8	112.4
45	10.7	110.3
46	8.6	108.2
47	6.4	106.2
48	4.3	104.1
49	2.1	102.0
50	0	100.0

Strength of Alcohol.	Volume of Water to add to 100 Vols.	Strength of Alcohol.	Volume of Water to add to 100 Vols.
Per cent	Per cent	Per cent	Per cent
100	107.4	74	50.3
99	105.6	73	48.1
98	102.7	72	46.0
97	100.4	71	43.9
96	98.1	70	41.8
95	95.9	69	39.7
94	93.6	68	37.6
93	91.4	67	35.4
92	89.2	66	33.3
91	87.0	65	31.2
90	84.8	64	29.1
89	82.6	63	27.0
88	80.4	62	25.0
87	78.2	61	22.9
86	76.0	60	20.8
85	73.8	59	18.7
84	71.7	58	16.6
83	69.5	57	14.5
82	67.4	56	12.4
81	65.2	55	10.4
80	63.1	54	8.3
79	60.9	53	6.2
78	58.8	52	4.1
77	56.7	51	2.1
76	54.5	50	0.0
75	52.4		

identical colours as possible. Assuming a 10 mm. layer of the standard has been used in the colorimeter, then the following are the amounts of furfural per 100,000 parts of *absolute* alcohol indicated by the following depths of the solution being found equal to the standard :—

mm.	Parts per 100,000 of absolute alcohol.
200	0.1
100	0.2
66.7	0.3
50	0.4
33.3	0.6
25	0.8
20	1.0
13.3	1.5
10	2.0
8	2.5
5	4.0

In carrying out these colorimetric processes for the determination of aldehydes or of furfural, it is well, as Vasey has pointed out, to subject the standards to exactly the same treatment as the sample under examination, by actually distilling the standard furfural solution under conditions identical with those used in the distillation of the sample.

J. T. Hewitt ("Jour. Soc. Chem. Ind.," Jan. 1902) prefers to distil the sample (unless it be colourless) nearly to the last drop, a little fresh pure spirit is poured into the distillation flask and the process repeated several times and the mixed distillates made up to a certain volume and then matched in glass cylinders by the standard solution as in the well-known process of "Nesslerizing". There is, however, a risk of furfural being actually formed during this repeated distillation, from the heated residue in the flask, and the results may be somewhat too high.

Determination of Esters.—The fact that alkalies act upon aldehydes renders it necessary to remove these if a correct determination of esters is required. This is best effected by boiling the alcohol under a reflux condenser for an hour with 3 per cent of meta-phenylene diamine hydrochloride. The liquid is then distilled, 90 per cent being collected and being made up to the original volume. To 100 c.c. of this liquid, a few drops of phenol-phthalein are added and decinormal baryta solution added to exact neutralization. Twenty-five c.c. of decinormal alcoholic solution of soda are then added and the whole boiled under a condenser for an hour. The excess of soda is then determined by titration with decinormal hydrochloric acid, each c.c. of alkali used being equivalent to 0.0088 gm. of ethyl acetate, in which form the esters are returned. From the alcoholic strength of the liquid the amount per 100,000 of absolute alcohol is calculated.

[The French official method takes no notice of the presence of aldehydes, but recommends that where the proportion is appreciable, the saponification should be by means of a standard solution of lime in sugar solution, which does not act appreciably on aldehydes.]

Determination of Higher Alcohols.—Whatever the exact nature of the alcohols present in what is usually termed the "fusel oil" of distilled spirits, or more correctly, the "higher alcohols," it is certain that isomeric amyl alcohols (principally 3 methyl-butanol 1, and 2 methyl-butanol 1) are the most important, and after these, normal iso-primary butyl alcohols. Any process of determining these alcohols is necessarily more or less empirical, and most processes described are now quite discredited. Schidrowitz and Kaye have exhaustively examined the more promising process and have shown that the German official process (Röse-Stutzer-Windisch) is useless in most cases; that Beckmann's process ("Zeit. Unter. Nahr. v. Genuss." II. 709; IV. 1057) is quite misleading; and that the French official process (Girard and Cuniasse), whilst giving fair results with brandy, is misleading in regard to whisky.

The process upon which most reliance is to be placed is that of Marquardt, as modified by A. H. Allen and slightly modified by Schidrowitz ("Jour. Soc. Chem. Ind.," 1902, 815). The details of the process are as follows:—

Two hundred c.c. of the spirit are boiled under a reflux condenser for an hour with 0.2 grm. of KOH, by which means acids are combined, and esters and furfural are decomposed. The liquid is now distilled until 180 c.c. have passed over, and steam passed through the residue till 300 c.c. are collected. The alcoholic strength of the liquid should be as near 50 per cent by volume as possible. If too low, it should be raised by the addition of *pure* alcohol. The exact volume of the liquid is now noted and 100 c.c. taken for the estimation. This is mixed with saturated brine until the mixture has a specific gravity 1.100. The mixture is then extracted with three successive quantities of 40 c.c. of carbon tetrachloride (which has been purified by washing with water, boiling with chromic acid mixture, washing with sodium bicarbonate solution and finally again with water until neutral. It is then distilled and ready for use). The carbon tetrachloride solution is shaken with 50 c.c. of a saturated solution of potassium sulphate, the dry carbon tetrachloride separated and filtered, and then oxidized with a solution of 5 grms. of potassium bichromate, 2 grms. of H_2SO_4 and 10 c.c. of water, for at least eight hours in a water bath. Any loss during the heating for eight hours is prevented by having the flask ground to fit the reflux condenser, the tube of which is fitted with the rod and disc condensing device. The liquid is then distilled, first over a Bunsen burner, and then with steam, until about 300 c.c. have passed over. The carbon tetrachloride may be separated and washed once with water, the washings being added to the main bulk of the aqueous liquid. The aqueous distillate is now titrated with decinormal baryta solution. Methyl orange is, according to Allen, first used as an indicator, the end reaction indicating the neutralization of the traces of free mineral acid (HCl) that may have been found. It is continued with phenol-phthalein, this result giving the amount of organic acids which are calculated to valeric acid, and thence to amyl alcohol (but see below). Each c.c. of decinormal baryta solution used for the neutralization of the or-

ganic acids, corresponds to 0.0088 grm. of amyl alcohol (or to 0.0074 grm. of butyl alcohol).

Schidrowitz and Kaye ("Analyst," xxxi. 181) have shown that the organic acids have some effect on methyl-orange, and that the apparent mineral acid value is not, at all events principally, due to mineral acid in reality. So long as the apparent mineral acid value does not exceed $\frac{1}{10}$ th of the total acid value it may be neglected. In doubtful cases, the chlorine should be determined gravimetrically, and the mineral acid as HCl deducted from the total acidity.

The same authorities have very exhaustively examined the Allen-Marquardt process, and have, by starting from weighed quantities of amyl and butyl alcohols, shown that so long as the higher alcohols are not present to the extent of more than 0.15 per cent—which is usually true for commercial spirits, the process yields exceedingly accurate results. When the amount is over 0.15 per cent, the oxidation should go on for ten hours; when over 0.3 per cent, the determination is not reliable.

Marquardt's original process possesses one useful feature. When the organic acids were distilled he warmed their aqueous solution with excess of barium carbonate for some time, filtered the solution, and evaporated the water and weighed the barium salts. If an indication of the nature of the acids—and therefore of the alcohols—be required, a determination of the amount of barium in the barium salts, by conversion into barium sulphate, will give the mean combining weight of the acids and thus indicate the nature of the alcohols. If mineral acids be actually present in the distillate, the necessary allowance for the barium chloride found must, of course, be made.

Bell some time ago suggested replacing the bichromate used in the Marquardt process by permanganate of potassium, but this was generally regarded as a retrograde step. Mitchell and Smith ("U.S. Dept. of Agriculture Bull." 122, 1909, 199) have again suggested this, and as the bulletins of the Department carry official weight in America, it is advisable to describe their process. The carbon tetrachloride solution of higher alcohols is placed in a separator with 10 c.c. of a 50 per cent solution of KOH and the mixture cooled to 0°. One hundred c.c. of a 2 per cent solution of potassium permanganate are placed in a flask, cooled to 0° and then added to the separator. The mixture is well shaken for five minutes and then set aside for thirty minutes at the laboratory temperature. One hundred c.c. of H₂O₂ solution which is rather stronger, relatively, than the permanganate solution are now placed in a 1000 c.c. flask, 100 c.c. of 25 per cent H₂SO₄ added, and the contents of the separator added with continual shaking. The separator is rinsed with water, which is added to the H₂O₂ solution. The excess of H₂O₂ is titrated with a standard permanganate solution (about 1 per cent). A blank experiment is carried out at the same time, and the amount of permanganate used for oxidation is noted. It is found that 1 grm. of permanganate oxidizes 0.475 grm. of propyl alcohol, 0.585 grm. of isobutyl alcohol, and 0.696 grm. of amyl alcohol.

In the same bulletin, processes due to Tolman and Hillyer are described as follows:—

(1) *Estimation of Colouring Matter*.—Fifty c.c. of the spirit are evaporated to dryness, the residue dissolved in 26.3 c.c. of 95 per cent alcohol and this solution diluted to 50 c.c. with water. Twenty-five c.c. of this solution are treated in a separator with 20 c.c. of a solution consisting of 100 c.c. amyl alcohol, 3 c.c. of syrupy phosphoric acid and 3 c.c. of water. The whole is well agitated and allowed to separate, three times. The aqueous layer is drawn off and diluted to 25 c.c. with 50 per cent alcohol. The colour of this solution is now compared with the colour of the other 25 c.c. of the original solution of the dried residue, which has not been treated with the amyl alcohol mixture. The percentage of the colour which has not been dissolved by amyl alcohol is thus obtained. This is stated by the authors to be due to added caramel. In the determination of the higher alcohols, these chemists prefer to determine the excess of bichromate left after oxidizing the carbon tetrachloride solution, by the liberation of iodine from potassium iodide and titrating this with standard solution of sodium thiosulphate. A blank experiment is carried out, and each c.c. of decinormal thiosulphate required by the blank in excess of the sample is equivalent to 0.001773 grm. of amyl alcohol.

The French official method for the determination of higher alcohols appears to give very fair results with brandy, although its indications with whisky are erratic. In spite of the fact that amyl alcohols are the most important of the higher alcohols naturally found in spirits, this process adopts iso-butyl alcohol for its standard solution. This solution contains 0.667 grm. of iso-butylic alcohol in 1 litre, the solvent being alcohol of 66.7 per cent strength.

One hundred c.c. of the brandy, etc., are distilled and the distillate adjusted to exactly 50 per cent alcoholic strength. This is placed in a 250 c.c. flask, 1 c.c. of pure aniline and 1 c.c. of syrupy phosphoric acid added, together with a few pieces of pumice stone. The liquid is gently boiled for an hour under a reflux condenser. It is then allowed to cool, and then distilled until 75 c.c. have passed over. This will, of course, be of 66.7 per cent alcoholic strength. To 10 c.c. of this 10 c.c. of pure colourless monohydrated H_2SO_4 (sp. gr. = 1.799) are added. The acid and alcohol are well mixed and heated to 120° for one hour in a chloride of calcium bath. Ten c.c. of the standard solution are treated in the same manner with sulphuric acid and the colours of the two liquids compared in a colorimeter. The standard solution corresponds to 0.667 grm. of iso-butyl alcohol per litre of 66.7 per cent alcohol, so that if the colour of the two liquids is identical, the amount of higher alcohols (calculated as iso-butyl alcohol) per 100,000 of absolute alcohol, would be 100. In the case of any material divergence in colour, the only correct method is to repeat the experiment with such a quantity of the alcohol as to give practically identical colours. Approximate results may, however, be obtained by constructing a curve from the following values, and by intercalating, the true result may be found. As the colours are not

directly proportional to the amounts of alcohols present, assuming 10 mm. of the sample matches 10 mm. of the standard solution, the value will be 100 parts per 100,000 of absolute alcohol; but

260	mm. matching 10 mm. of the standard=					10 per 100,000
83	"	"	"	"	"	= 20 "
33	"	"	"	"	"	= 40 "
19.5	"	"	"	"	"	= 60 "
13.2	"	"	"	"	"	= 70 "
4.4	"	"	"	"	"	= 200 "
3.7	"	"	"	"	"	= 250 "
3.1	"	"	"	"	"	= 300 "
2.3	"	"	"	"	"	= 400 "

The empirical nature of this process is very apparent, when one remembers that no two alcohols give exactly the same colour with sulphuric acid, and consequently no two "fusel oils" can be expected to give similar results. Schidrowitz has stated that this process gives fair results with brandy, but not with whisky. Certainly the process based on the Allen-Marquardt process gives the best result of any so far devised. The French official process, giving results expressed in terms of butyl alcohol, is always below the truth—since amyl alcohol is the predominant alcohol present. The cardinal defects in this process are discussed by Schidrowitz and Kaye in the "Analyst," xxxi. 185.

Vasey recommends, as an approximate method, enabling a fair distinction to be drawn between genuine distillates of the grape or malt and mixtures of these with silent spirit, the following process (which in the author's experience, although giving careful results, does not enable the discrimination claimed for it to be made).

Ten c.c. of a distillate from spirit whose alcoholic strength has been adjusted to exactly 50 p.c. are taken and to it are added 10 c.c. of monohydrated H_2SO_4 specific gravity 1.794, in a test tube 6 inches long by 1 inch. The contents of the tube are mixed by shaking, and a small piece of glass tubing is dropped into the mixture. It is then heated over a flame, and directly bubbles of steam arise from the fragments of glass tubing, the test tube is withdrawn from the flame for twenty seconds and then returned, and so on, until five minutes have elapsed. The tube is then cooled, and the volume made up to 20 c.c. with 50 per cent alcohol. Ten c.c. of a standard solution of isobutyl alcohol (0.2 per cent in 50 per cent alcohol) are heated in the same manner, and the colours compared, and the approximate amount of higher alcohols thus calculated as isobutyl alcohol.

Bedford and Jenks have proposed a process depending on the formation of nitrous acid by nitration of the alcohols, and a determination of the iodine liberated by the acid from potassium iodide, but as neither this process nor that of Beckmann give nearly so concordant or accurate results as the Allen-Marquardt, they are not described. The same is true of Röse's process, which is recognized officially in Germany, and which depends on the increase in volume of a measured volume of chloroform when shaken with a measured quantity of the alcohol at 30 per cent strength and a small quantity of sulphuric acid, under rigidly defined conditions.

In addition to the above determination, it may be necessary to examine the spirit for methyl alcohol. This can be done in any of the usual methods (see p. 280), but the following may be quoted as being the official method in France.

Fifteen grms. of potassium bichromate are dissolved in 130 c.c. of water, and 70 c.c. of H_2SO_4 (1 to 5 of water). Ten c.c. of alcohol of about 90 to 95 per cent or an equivalent amount of a diluted spirit to be tested, are added. The mixture is allowed to stand for twenty minutes. It is then distilled, the first 25 c.c. being rejected and the next 100 c.c. collected. To 50 c.c. of this 1 c.c. of dimethyl-aniline is added, and the mixture kept at 70 to 80° for three hours with continual shaking, in a well-stoppered bottle. It is then rendered distinctly alkaline with caustic soda solution (about 5 c.c. of a 16 per cent solution) and 30 c.c. are distilled off to drive off dimethyl-aniline. To the residue in the flask, 25 c.c. of water, 1 c.c. of acetic acid, and 4 or 5 drops of water containing lead dioxide in suspension. The solution must be acid. In the presence of methyl alcohol, the liquid becomes blue, the colour being intensified by boiling. Ethyl alcohol becomes blue, changing at once to green, then to yellow, and becoming colourless on boiling.

It must be obvious to the meanest understanding that so long as the determination of the groups of bodies as outlined above, be considered as a standard of purity, the addition of such bodies to what may fairly be called "neutral spirit" can be practised, and so succeed in deceiving the analyst.

So far, all researches on the analysis of genuine potable spirits come to this, and to no more. Natural genuine potable spirits contain certain quantities of acids, esters, aldehydes and higher alcohols. With the determination of these, the resources of the analyst finish. "Silent" spirit—such as potato alcohols, contain very little of such bodies. All these bodies are commercial products easily obtainable. If such bodies are added to silent spirit in due proportions—there are no chemical means available to decide whether they are naturally present or have been added. It is true that the *absence* of due proportions of such bodies may *prove* adulteration, but presence of them is no more than presumptive evidence of purity. The honest analyst must stop at that until further developments may arise to assist him.

Hence the absolute necessity of the opinion of the trade expert in conjunction with that of the analyst.

The analyst, however, can obtain much useful information by cultivating the sense of taste, and by making up numerous mixtures of such secondary constituents, and thus enabling himself to reject artificial spirits, not only by the results of his analysis, but also by the use of the palate. The great danger to be avoided is in too implicit reliance on arbitrary chemical standards.

Assuming that a spirit is genuine and not artificially prepared with silent spirit and added secondary constituents, the following remarks as to the interpretation of results obtained as above may be useful: A genuine brandy will rarely contain less than 280 parts

per 100,000 of absolute alcohol of all secondary constituents calculated in the manner described above. Very old spirits may show a very much higher amount than this. The proportion of higher alcohols to esters in brandy rarely if ever varies outside the limits of from one to two of alcohols to one of esters. In general, secondary products increase with age, but the furfural diminishes. Assuming no artificial products have been added, it is safe to say that "silent" spirit contains very little esters, and a low ester value is indicative of adulteration.

WHISKY.

The question as to what whisky is has aroused a good deal of discussion during the past three or four years. On the one hand it was contended that only the product of cereals distilled from a pot still could properly be described as whisky, since the patent still eliminated the greater portion of the secondary constituents of the fermented liquid. On the other hand, it was contended that the term whisky was equally applicable to pot and patent still products. Some went so far as to allow the spirit distilled from potatoes to be termed whisky. Indeed, a statement to this effect appears so recently as in the 1901 edition of Vol. I of Allen's Commercial Organic Analysis (p 143). The question has now been settled, for this country at all events, by the publication of the Royal Commission on whisky and other potable spirits, 1909. The following remarks cover the whole of the question from the point of view of the legal standard. The Commissioners state:—

"The evidence which we received, shows that such spirits have been frequently described as 'whisky' by distillers and traders since the patent still came into use; and that for many years a section of the public, particularly in parts of Scotland and Ireland, has recognized patent-still spirit without admixture under the name of whisky, and has purchased it as whisky, no attempt being made by distillers or vendors to conceal the method of distillation. Moreover, spirit produced in the patent still, as we have shown, has long been employed for blending with or diluting whiskies of different character and distilled in different forms of still. This has been by far its largest use, and most of the whisky now sold in the United Kingdom contains in greater or less degree spirit which has been obtained by patent-still distillation.

"Again, apart from the fact that pot stills differ so much that a comprehensive legal definition would be difficult to frame without either excluding certain types of still which are now commonly recognized as pot stills, or including other types which are not now looked upon as legitimate variations of the pot still, there are strong objections to hampering the development of an industry by stereotyping particular forms of apparatus.

"Finally, we have received no evidence to show that the form of still has any necessary relation to the wholesomeness of the spirit produced.

"For these reasons we are unable to recommend that the use of

the word 'whisky' should be restricted to spirit manufactured by the pot-still process.

"The taste of the consumer creates the demand which ultimately controls the trade. The public purchases the whisky that meets its taste, and the blender must satisfy that taste or lose his trade. It is not for the State to say what that taste ought to be.

"In our opinion, the use of the term 'Scotch' and 'Irish' as applied to whisky cannot be denied to any whisky distilled in Scotland and Ireland respectively.

"Our general conclusion, therefore, on this part of our inquiry is that 'whisky' is a spirit obtained by distillation from a mash of cereal grains saccharified by the diastase of malt: that 'Scotch whisky' is whisky, as above defined, distilled in Scotland, and that 'Irish whisky' is whisky, as above defined, distilled in Ireland."

The general remarks with reference to the question of the secondary constituents of brandy, and the methods of analysis, apply in general also to whisky. Whisky may be diluted with water to a minimum strength of 25° under proof and sold as whisky without an offence being committed under the Sale of Food and Drugs Acts.

The solid residue in whisky averages about 0.01 per cent, or from 0.004 per cent to 0.038 per cent. The mineral matter varies from 0 to 0.02 per cent. This residue is, of course, derived from the casks in which the spirit is stored.

For a series of analyses of whiskies of all types, reference should be made to papers by Schidrowitz and Kaye ("Jour. Soc. Chem. Indus." 1902 and 1905).

Parts per 100,000 of Absolute Alcohol.						
	Volatile Acids.	Aldehydes.	Furfural.	Esters.	Higher Alcohols.	Observers
New whisky, Scotch . . .	25.4	11.4	6.2	61.9	199.4	Schidrowitz
Whisky 5 years old Scotch	20.1	21.3	3.7	109.4	148.3	"
" 9 " " " . . .	65.4	28.0	3.9	75.6	239.7	Girard
Irish whisky, new " . .	20.88	6.52	0.43	7.65	174	Vasey
" 10 years old " . . .	51.9	14.41	3.46	30.44	259.5	Cuniasse
Maize whisky " . . .	14.3	3.0	3.4	90	263.3	Schidrowitz
Rye " " " " . . .	13.5	12.8	—	69.4	76.2	"
Malt " " " " . . .	28.1	13.2	1.8	112.7	182	"
All malt pot still " . .	19.5	12.6	2.2	98	280	Parry
All malt patent still " .	9	6.5	0.4	48	120	"

In dealing with the question of the estimation of the higher alcohols in whisky by the French official process, Schidrowitz states that it is useless in the case of this spirit, since amongst other reasons, the higher alcohols of whisky consist of more than one individual (probably much more variable than in the case of brandy) and as each

alcohol gives a very different colour with sulphuric acid, no quantitative result can be of value.

The analyses of whisky given on page 302 will show the average amounts of secondary constituents, of pure whisky. No differentiation is here made, except in one case, between pot and patent still whiskies; analyses of "silent spirits" will be found on p. 290.

American whisky has been exhaustively examined by Crampton and Tolman ("Jour. Amer. Chem. Soc." 1908 **30**, 98).

The results of their analyses of numerous samples of rye and Bourbon whisky are as follows:—

(1) RYE WHISKY.

Grams. per 100 Litres of Proof Spirit.							
	Proof Spirit Value.	Extract.	Acids.	Esters.	Aldehydes.	Furfural.	Fusel Oil.
Maximum	132	339	112	126.6	26.5	9.2	280.3
Minimum	100	5 (new)	12	4.3	0.7	trace	43.7

The samples varied from quite new to eight years old.

(2) BOURBON WHISKY.

Maximum	124	326	91.4	93.6	28.8	10.0	241.8
Minimum	100	4.0	7.2	10.4	1.0	trace	42.0

In interpreting the results of the analysis of potable spirits, it must be remembered that it is only in certain cases that positive deductions can be drawn. Hehner has gone so far as to say the chemical analysis cannot decide, but that the expert taster must be called in. There is no doubt that in the large majority of cases this is true, but where a brandy, for example, shows secondary constituents *appreciably* below 250 parts per 100,000 of absolute alcohol or esters much below 80 (a low figure in itself) it is quite fair to pronounce it as mixed with silent spirit—unless it can be shown that it was in fact made in some parts of the world where patent stills were used.

Equally, a genuine whisky will almost invariably show a considerable excess of higher alcohols over esters, whereas in Jamaica rum the esters are always far in excess of the higher alcohols.

The author cannot agree with the general deduction of Vasey as to the utility of the analysis of these spirits (Vasey, "Analysis of Potable Spirits," *passim*). The analyst cannot be too careful in his deduction, and except where the figures are obviously those outside

the limits of a pure spirit, should hesitate to condemn a sample without joining his opinion with that of an expert spirit taster.

RUM.

By rum, the spirit distilled from fermentation products of the juice of the sugar cane was at one time invariably understood. In old works of reference it is always so described, and in an old volume of the eighteenth century (Shaw's "Essay on Distilling") the following remarks occur: "Rum is usually very much adulterated in England; some are so barefaced as to do it with malt spirit; but when it is done with molasses-spirit, the tastes of both are so nearly allied, that it is not easily discovered." Up till recently much so called "rum" was to be found in commerce which was either made from beet sugar molasses, or from neutral spirit which is flavoured with artificial rum essences.

So far as this country is concerned the word rum may be taken as indicating the product as defined in the report of the Royal Commission on Whisky and other potable spirits (1909). In the course of their report, the Commissioners state that it was suggested during the inquiry that the principal cause for the difference in flavour between rums produced in various places lies in the methods of fermentation used rather than the process of distillation. According to the evidence, there are two distinct types of rum, Jamaica rum being representative of the first, and Demerara rum of the second.

The Commissioners see no reason, however, to deny the name of rum to either of these types. They consider that the definition of rum as "a spirit distilled direct from sugar-cane products in sugar-cane growing countries," submitted by Mr. Aspinall, on behalf of the West India Committee, fairly represents the nature of the spirit which a purchaser would expect to obtain when he asks for "rum". The Customs already recognize the distinction between "rum," "rum from Jamaica," and "imitation rum," and they consider that this differentiation should be continued.

The characteristic flavour of rum is due to a mixture of esters in which butyric and acetic esters of ethyl alcohol predominate. Artificial mixtures of esters of this type are regular commercial articles, and are used largely in the preparation of factitious rums. The remarks made under brandy as to the limits to which chemical analysis can go to apply equally to rum, as, of course, do the various processes there described.

Rum may be sold as such, when diluted with water, provided the strength be not below 25° under proof.

The characteristic feature of the secondary constituents of genuine rum is the large excess of esters over higher alcohols. The following analyses of genuine rums are due to Collingwood Williams. They are all genuine rums, the flavoured samples not being those to which a small quantity of fruity flavouring matter has been added—such as, possibly, a trace of pineapple, etc.—as is sometimes stated, but are samples whose flavour is developed by a special method of fermentation.

The Jamaica rums are placed in the order of their quality as judged by the smell of a diluted sample.

COMMON CLEAR JAMAICA RUMS.

Parts per 100,000 of Absolute Alcohol.					
No.	Volatile Acid.	Esters.	Higher Alcohols.	Furfural.	Aldehyde.
3	76	557	82	1·8	7·5
10	74·5	565	—	3·5	7·5
6	21	332	—	1·0	20·0
13	146	297	—	3·7	10·0
2	55	310	—	3·3	9·0
5	62	216	—	6·3	15·0
14	72	355	—	2·3	12·5
28	61	164	—	—	—
19	61	351	—	4·0	17·5
9	55	480	80	7·0	5·0
7	60	303	120	2·7	25·0
11	60	308	—	9·0	20·0
15	76	372	—	4·5	18·0
16	52	516	—	6·0	15·0
18	41	321	75	4·5	6·0
17	31	388	—	4·5	17·5
20	27	88	—	—	—
8	46	266	—	11·5	20·0
4	61	181	—	4·6	30·0
12	56	211	—	3·2	25·0
Average	60	333	—	4·6	75·5
Highest	146	565	—	11·5	30·0
Lowest	21	88	—	1·0	50·0

FLAVOURED JAMAICA RUMS.

No.	Volatile Acid.	Esters.	Higher Alcohols.	Furfural.	Aldehyde.
25	137	981	—	—	—
1	122	1204	—	2·9	13
24	116	552	—	12·0	15
23	93	787	—	2·7	17·5
22	39	599	—	4·5	37·5
21	75	1053	—	3·6	12·5
26	109	866	—	4·1	—
27	53	391	—	—	—
Average	93	805	—	5	19

DEMERARA RUMS.

No.	Volatile Acid.	Esters.	Furfural.	Vat Still.	Volatile Acid.	Esters.
1	75	53	2.7	Average Continuous	33.1	69.9
2	71	48	1.6		18.4	44.4
3	34	37	0.6			
4	33	96	2.6			

The following are confirmatory analyses by various observers :—

Samples.	Volatile Acids.	Esters.	Higher Alcohols.	Furfural.	Aldehydes.	Observers.
Jamaica rum	28	399	90.6	2.8	8.4	Vasey
" " " "	176	443	93.9	2.9	22.1	Girard
(Average of 10 samples)	48	338	84.0	3.2	11.9	Parry

Bonio ("Annales Falsific," 1909, 12, 521) states that the better-class rums (Martinique rums) usually contain more secondary constituents than lower-grade samples, but that the ratio of the esters to the free acids and the higher alcohols is more important than the total amount.

He gives the following analyses :—

Per 100,000 of Absolute Alcohol.									
Molasses Rum.	No.	Volatile Acids.	Aldehydes.	Esters.	Higher Alcohols.	Furfural.	Total.	Fixed Acids.	Ratios of Esters to Higher Alcohols.
High grade	1	201.3	92	443.5	67.5	8.8	813	2.2	6.6
	2	201.0	59	91.5	385.0	5.3	742	0.46	0.24
	3	174.0	32	93.2	425	11.0	735	0.54	0.22
	4	165.3	34.5	61.6	339	0.9	601	0.37	0.18
Average	5	173.2	20	82.7	244	0.5	520	0.48	0.34
	6	145.2	23	117.9	167	6.3	459	0.81	0.71
	7	196.6	16.3	95	97	3.8	409	0.48	0.98
Low grade	8	158.5	14.6	89.7	143	0.1	406	0.57	0.63
	9	53.5	10.4	51	280	0.7	396	0.95	0.18
	10	60	10.0	77	300	1.4	448	1.28	0.26
Sugar cane juice rums	11	80.7	10.0	63.3	256	1.5	422	0.79	0.25
	12	80.8	19.0	74.0	243	0.8	418	0.91	0.30
	13	83.4	18.6	68.6	214	1.8	390	0.82	0.31
	14	42.4	17.3	61.6	283	1.2	406	1.45	0.22

These figures apply to Martinique rum, but it is not easy to understand them, as Jamaica rums may be said to practically invariably contain considerably more esters than higher alcohols. It is probable that if these determinations had been made by the Allen-Marquardt process, the results might have been very different.

Simon (*"Annales Falsific,"* 1909, **12**, 494) considers that the quality of rum is mainly dependent on the esters, but that the free acids are important as regards its flavour. In Martinique rum he also finds the alcohols are frequently higher than the esters.

Micko (*"Zeit. Untersuch. Nahr. Genuss,"* 1908, **16**, 433) states that he has found a peculiar, typical aromatic substance in Jamaica rum, which is absent from artificial rums and also from rum made in Europe from molasses. He states that by distilling a mixture of 200 c.c. of rum and 30 c.c. of water, and collecting the distillate in fractions of 25 c.c. each, the aromatic substance in question is found in the fifth or sixth fraction. But as he has not characterized the body other than by describing it as "aromatic" it is of little assistance to the analyst at present.

The solid residue of rum averages 0.3 to 0.5 per cent.

The author is entirely at variance, as are all other observers in Europe, with the standards adopted by the Joint Committee of the American Association of Official Agricultural Chemists and of State and National Food and Dairy Departments, which require that the principal part of the secondary constituents of rum should be higher alcohols calculated as amyl alcohol—whereas they are in fact principally esters.

GIN.

Gin is a more or less neutral alcoholic liquid, flavoured with juniper and sometimes with other substances, and frequently sweetened by the addition of sugar. The definition accepted by the Royal Commission above referred to is as follows: "Gin may be defined as a spirit distilled from grain doubly rectified, and then flavoured by distillation with juniper berries and other herbs. Geneva, also called Hollands, is a foreign spirit imported into this country: it resembles gin, inasmuch as in both cases the genuine article is made from grain only, and flavoured with juniper."

It may not be out of place to reproduce the opinions held in regard to what gin should be in the middle of the eighteenth century as stated in Shaw's "Essay on Distilling". Dr. Chambers in his "Encyclopedia" (1783) states that "Geneva or gin, is a popular name for a compound water which is, or ought to be, procured from the berries of the juniper tree, distilled with brandy or malt spirits. The word is from Genevre, the French name of the Juniper berry." He then quotes from Shaw as follows:—

"The best Geneva we now have is made from an ordinary spirit distilled a second time with an addition of some juniper berries: but the original liquor of this kind was prepared in a very different manner" Shaw then describes how the berries were added to the

malt in the grinding and observes that "the spirit thus obtained was flavoured *ab origine* with the berries and exceeded all that could be made by any other method. Our common distillers leave out the juniper berries entirely from the liquor they now make and sell under that name. Our chemists have let them into the secret that the oil of juniper berries and that of turpentine are very much alike in flavour, though not in price: and the common method of making what is known in London as Geneva is with a common malt spirit and a proper quantity of oil of turpentine distilled together."

Although the Royal Commission's definition speaks of this spirit being distilled with *juniper berries*, it is difficult to conceive that a spirit which had been made with a grain spirit and *essential oil* of juniper would ever be condemned as not being gin. Gin may be diluted with water to a minimum strength of 35° under proof, and sold as gin without an offence being committed under the Sale of Food and Drugs Acts. Various aromatic flavourings are used in certain varieties of gin, including cardamon, coriander, angelica, *acorus calamus*, grains of paradise, etc.

It must be definitely stated that the standards for secondary constituents as indicated for brandy, rum or whisky have *no meaning whatever* as applied to gin. Gin stands quite alone in this respect, and the analyses quoted by Vasey ("The Analysis of Potable Spirits," p. 25) are quite useless. They are as follows:—

Parts per 100,000 of Absolute Alcohol.						
	Volatile Acids	Aldehydes.	Furfural.	Esters.	Higher Alcohols.	Observers.
1	nil	1.78	nil	37.28	83.66	Vasey
2	40.4	9.90	0.3	18.50	97.00	Girard

Gin is essentially a neutral spirit, flavoured with the volatile constituents of juniper berries—and to a lesser extent with those of other aromatics. It is open to any maker to flavour heavily or lightly, and the addition of many times as much flavouring in one case as in the other, in no way alters the legal character of the gin. But, as the essential nature of gin is its juniper flavour, and as this is due to essential oil of juniper, it is obvious that none of the groups of compounds enumerated for other spirits is the essential secondary constituent of gin. Juniper oil consists almost entirely of terpenes and sesquiterpenes, which would possibly be returned as "higher alcohols" although not in the least related to them.

It should not be forgotten that the "secondary constituents" of the other potable spirits are the results of natural processes in the formation of the alcohol, and, subject to the necessary limitations above given under "Brandy" can be dealt with as "standards," whereas in the case of gin, the secondary constituents are deliberately,

but legitimately, added, and to what extent is merely a matter of taste.

Apart from the alcoholic strength, gin should be tested for methyl alcohol and if required, the sugar, when present, determined in the usual manner in an aqueous solution of the solid residue left on evaporation. If an extract be made in the same manner as by the Allen-Marquardt process, but with the lightest petroleum ether obtainable, and the solvent allowed to evaporate, the taste of the residue will afford considerable information as to the nature of the essential oil present. If 500 c.c. be so treated, usually at least 0.5 grm. can be obtained, which is sufficient for the determination of the refractive index. The author has examined ten authentic samples in this manner and found the refractive index to never fall below 1.4750, usually about 1.4770 at 20°. If turpentine be used, the refractive index will fall to 1.4725 or lower, but the author has never met a case where it has fallen so low.

WINE.

Wine, without further qualification, is understood to be the product of fermentation of the juice of the grape, with at most such additions as are essential to its preservation. Public taste in various countries has to a great extent altered the primitive meaning of the word, in the sense that a wine containing more alcohol than a natural fermentation of grape juice will yield, is demanded. Hence many of the wines of to-day are fortified or increased in alcoholic strength by the addition of alcohol. Where such fortification is effected by the addition of brandy—a wine product—the wine may be correctly described as such, but where alcohol derived from another source is used, the finished product is not in the proper sense a pure wine.

The manufacture of wine is, of course, a subject which would require a special volume to itself, but from a broad point of view it consists in the conversion of the saccharine matter in the expressed juice of the grape by the action of a yeast, *Saccharomyces ellipsoideus*, and allied species, into other constituents, of which ordinary alcohol is the principal. At the same time, the nitrogenous constituents of the grape juice assist in the feeding of the organisms, and are thus changed in their nature, and other subordinate changes in other constituents are effected at the same time. Details of the manufacture of wine, however, do not come within the scope of the present work.

Classification of Wines.—Wines may be divided into numerous classes, such as *red and white*, depending on the colour of the grape used, or upon the use or rejection of the skin of the black grape; dry or sweet, depending on the absence or presence in large amount of sugar in the finished product; still or sparkling, dependent on the absence or presence of carbonic acid gas; and—of course—natural or fortified, dependent on the absence or presence of added alcohol.

The distinction of wines, however, into geographical groups, such as Burgundy, port, sherry and so on, is a matter which has more importance in connexion with the administration of the Merchandise Marks Act than with that of the Food and Drugs Acts. Such wines

can be instantly distinguished by the palate ; so that the sale of port for Burgundy would be an absurdity ; but as port is recognized as the product of Portugal shipped from the neighbourhood of Oporto, the sale of a Spanish wine under the name of port (a not uncommon proceeding) is an offence under the Merchandise Marks Act, since it should be described with the qualification Spanish port or Tarragona port. The wine produced in the Burgundy district has, equally, earned the right to the sole use of its geographical description, and wines of the Burgundy type produced in California or Australia should be described as such. Whether the name claret is restricted legally to a wine produced in the Bordeaux district, is less certain.

The wine country of the world is undoubtedly France. Germany produces excellent wines ; Portugal and Spain produce port and sherry respectively. Austria and Italy produce much excellent wine ; and during the past twenty years Australia and California have given to the world wines of very high grade indeed. All these are the true product of the grape. But so long as properly qualified, there is no reason why the word wine should not be used for the product of fermentation of other fruits, such as, for example, the well-recognized "British Wines". These are produced by the fermentation of various fruits and are never suggested as being the product of the fermentation of grape juice.

At the outset it is necessary to inquire what are the legitimate additions to the juice of the grape in the manufacture of wine. In this country there are no statutory definitions, so that we naturally turn to the home of the industry to see what legal restrictions exist in the matter.

In this connexion it is easy to see that the addition of a saccharine solution to the juice of the grape will result in the formation of a correspondingly increased amount of fermented alcoholic liquid, but which will be proportionately deficient in the minute quantities of secondary constituents which discriminate wine from a mere solution of alcohol.

Every precaution is taken in France to preserve the good character of its wine industry. By the law of 29 June 1907, amending previous laws on the subject, every vine grower must declare his acreage and his average produce, and the quantity of sugar which he may have delivered to his home may not exceed 20 kilograms per head of his establishment for personal use, subject to a heavy penalty. This law is intended to entirely prevent the addition of sugar to the must to increase the amount of "wine" obtained.

A decree dated September, 1907, issued under statutory authority, defines clearly what may be sold as wine in the republic of France.

All beverages sold as wine must be derived exclusively from the fermentation of fresh grapes, or the juice of fresh grapes. The following "manipulations" are not to be considered as illegal in any way in the manufacture of wine.

- (1) Blending of wines.
- (2) Freezing of wines to increase their alcoholic strength.
- (3) Pasteurization.

(4) Clarification by the use of well-known agents such as albumen, fresh blood, casein, gelatine.

(5) The use of tannic acid in the amount necessary to effect clarification by means of albumen or gelatine.

(6) Decoloration of white wines by means of charcoal.

(6) The use of SO_2 resulting from the combustion of sulphur, and of alkaline bisulphites. The amounts which may be employed are such that the wine shall not retain more than 350 mg. per litre of SO_2 free and combined. In no case shall alkaline bisulphite be employed to a greater extent initially than 20 grms. per hectolitre.

In regard to the treatment of the grape-juice or must, a little tartaric acid may be added to musts not sufficiently acid, as well as selected yeasts where necessary.

In regard to sparkling wines, a further rule is in force, that, whilst the artificial aeration of sparkling wines by means of CO_2 is allowed, such practice must be indicated on the label, by the use of the word "fantasie," so that the resulting wine must not be simply described as "sparkling".

The Adulteration of Wine.—Wine is adulterated in the following manners:—

(1) By the addition of saccharine matter to the must in order to increase the amount of alcohol produced. Water is generally added at the same time, so as to increase the volume of the wine also.

(2) By the addition of fermented liquors from other fruits than the grape, and as these are usually prone to undergo acetous fermentation, antiseptics are often added as well.

(3) By the addition of ordinary alcohol.

(4) By dilution with water.

(5) By the addition of extraneous colouring matter, especially where a pale-coloured liquid has been used as the adulterant.

(6) By plastering, that is by adding more than a small quantity of plaster of paris, in order to fine the wine and remove tartrates.

Before passing on to the question of the analysis of wine, a few words on natural and fortified wines may not be out of place. A natural wine is the product of the fermentation of the pure juice without the addition of sugar or alcohol. The fermentation has gone on until either the whole of the sugar has been used up, or till the nitrogenous food for the yeast has been exhausted, or until the alcoholic strength is such as to check further growth of the yeast. With an alcoholic strength of 14 per cent to 14.5 per cent by weight, no further fermentation due to yeast can take place, so that this figure may be taken as the highest limit for a natural wine. Fortified wines have frequently received the addition of alcohol before fermentation has finished, so that a wine of this type would be sweet on account of a large amount of grape sugar left in the wine. Sparkling wines may be perfectly natural, having been bottled before fermentation has finished, or they may be fermented to their full extent and then bottled with a little sugar to induce a secondary fermentation in the bottle. Or they may be changed artificially with carbonic acid gas. A dry wine has been allowed to ferment to the fullest ex-

tent, even perhaps with the addition of a little nitrogenous matter such as gelatine or albumen. It contains practically no sugar.

The analysis of wine is limited in value and rarely affords more information than that which may establish the purity or otherwise of a given sample. Speaking broadly, the analyst obtains no results which enable him, *qua* analyst, to pass an opinion as to the *quality* of a pure wine. This is essentially a matter of flavour and bouquet, and requires the experience of a trained wine taster. It is true that one may discriminate between a highly acid hock and one much less acid, or a claret containing enough tannin to feel rough to the palate, and one which is practically free from tannin, but excluding such simple cases, there is no guide to the analyst which will allow a discrimination between a claret worth 2s. and one worth 20s. a bottle, or a port 5 years old and one 50 years old. It is necessary, however, to understand the general character of the principal groups of wine, in order to be able to correctly interpret the results of analysis. The following are the chief types of wine in general use in this country. A number of typical analyses are appended.

(1) *Claret*.—As generally understood, claret is a red wine of deep colour and low alcoholic strength—from 7 per cent to 11 per cent by volume being the usual amount. It is made in various districts in the South of France, the Bordeaux district being the principal. *Sauternes* may be taken as typical white clarets, the grapes being usually grown in the Gironde district in the neighbourhood of Bordeaux. Red clarets contain very little sugar: white clarets are frequently sweet.

(2) *Burgundy*.—This is a red wine grown in the Burgundy district (Côte d'or, Saône et Loire, and the Yonne). It resembles clarets in its general character, but is of a different bouquet, rather fuller-bodied and usually of slightly higher alcohol content. *Chablis* is the type of a white Burgundy.

(3) *Port* is a Portuguese red wine, practically always fortified, containing from 15 per cent to 22 per cent of alcohol by volume and from 3 per cent to 7 per cent of sugar. It is thus a typical sweet wine. Tarragona port is a wine of similar type made in Spain. Port derives its name from Oporto, whence it is shipped.

(4) *Sherry*.—The "wine of Xeres" is a Spanish wine, varying in colour from very pale yellow, to a deep brown. It is a wine which may be either dry or sweet, the sugar varying from almost nil to a quite considerable amount. Its characteristic bouquet is dependent on its ethers, amongst which nitrous ethers are to be found.

(5) *Hock and Moselle* are German wines, produced in the Rhine and Moselle districts respectively. They are characterized by the very small amount of sugar they contain, so that they are often thought to be considerably more acid than French wines. This is not usually the case, however. The alcohol content varies from 8 per cent to 12 per cent by volume. Red German wines are not drunk in this country to any large extent.

(6) *Champagne*.—The wines produced in the old district of champagne—now covering the departments of the Ardennes, the Marne, the Aube and the Haut Marne, are, of course, very varied in character,

and are generally considered amongst the best class of their type. It is the *sparkling* wine of this district, however, that is meant when champagne is referred to in this country. This is a white wine which is allowed to undergo some fermentation in bottle, frequently assisted by the addition of a little sugar. It is a wine containing a fair amount of sugar—sometimes, in very dry wines, only small quantities are present—and from 9 per cent to 13 per cent of alcohol by volume.

(8) *Madeira*, made in the neighbourhood of Madeira, and *Marsala* made in Sicily, are very similar wines of the sherry type, but containing ethers which give to the wine a characteristic flavour. Both are almost invariably fortified and contain 18 per cent to 22 per cent of alcohol by volume.

Italy and Hungary produce excellent wines, more or less assimilating to the above types and similar wines are produced in Australia and California. Where custom has established a name for the wine of a given country, there is now no doubt that it is an offence under the Merchandise Marks Acts to apply that name to the wine of another country without a geographical qualification.

The following are typical characters of the principal varieties of wine :—

	Sp. Gravity.	Alcohol by Volume.	Solid Residue.	Total Acids as Tartaric.	Sugar.	Ash.
		Per cent	Per cent	Per cent	Per cent	Per cent
Claret	0.990 to 1.025	7 to 11	2.2 to 3.0	0.4 to 0.8	0.1 to 0.8	0.18 to 0.3
Burgundy	0.990 „ 1.035	8 „ 12	2.2 „ 3.6	0.4 „ 0.8	0.1 „ 0.9	0.18 „ 0.3
Port	0.990 „ 1.050	15 „ 22	5 „ 14	0.3 „ 0.7	3 „ 9	0.2 „ 0.4
Sherry	0.980 „ 1.020	15 „ 20	2.0 „ 5.5	0.35 „ 0.7	2 „ 5	0.2 „ 0.5
Hock	0.988 „ 1.010	8 „ 12	1.8 „ 3.5	0.4 „ 0.9	0 „ 0.2	0.18 „ 0.4
Moselle	0.985 „ 1.015	8 „ 12	1.8 „ 4	0.4 „ 0.9	0 „ 0.4	0.18 „ 0.4
Champagne	1.040 „ 1.060	9 „ 13	10 „ 19	0.5 „ 0.8	8 „ 17	0.1 „ 0.2
Madeira	0.995 „ 1.010	18 „ 22	4.5 „ 7	0.4 „ 0.6	3 „ 5	0.3 „ 0.5

THE ANALYSIS OF WINE.

In examining wine, the following determinations are made if the fullest information is desired :—

Specific gravity.
Alcohol.
Fixed residue.
Mineral matter.
Sugar and polarization value.
Total acidity—free and volatile.
Glycerine.
Sulphates.
Sulphurous acid.
Added colouring matter.
Tartaric acid.
Tannic acid.
Salicylic acid.
Succinic acid.
Saccharin.

The following represent the composition of a number of samples of wines of various origins.

IN GRMS. PER 100 C.C.

	Specific Gravity.	Alcohol per cent.	Extract.	Mineral Matter.	Phosphoric Acids.	Tartaric Acid.	Sugar.	Glycerine.	Tannin.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
French red wine									
Minimum	0.9890	6.4	1.96	0.190	0.015	0.38	0.11	0.57	0.11
Maximum	1.0401	9.3	14.20	0.300	0.062	0.78	0.84	1.04	0.30
Average	0.9982	7.8	2.56	0.248	0.030	0.57	0.30	0.73	0.18
French white wine									
Average	0.9963	10.3	3.03	0.250	0.032	0.66	—	0.97	—
Swiss red wine									
Average	0.9963	8.0	2.31	0.220	0.030	0.79	0.17	0.61	0.20
Swiss white wine									
Average	0.9904	7.6	1.860	0.244	0.030	0.43	0.07	0.64	—
Tyrol red wines									
Minimum	0.9905	6.7	1.50	0.182	0.017	0.48	—	0.41	0.08
Maximum	1.0140	11.0	6.55	0.269	0.055	0.85	—	1.14	0.27
Average	0.9940	9.0	2.34	0.222	0.027	0.62	—	0.65	0.17
Tyrol white wine									
Average	0.9927	8.8	1.87	0.175	0.022	0.59	—	0.65	—
Austrian white wine									
Minimum	0.9918	5.8	1.43	0.144	0.024	0.45	—	0.44	—
Maximum	0.9986	11.4	3.91	0.311	0.048	1.04	—	1.01	—
Average	0.9949	7.9	2.13	0.189	0.034	0.67	—	0.68	—
Austrian red wine									
Average	0.9958	8.4	2.54	0.241	0.037	0.62	—	0.81	0.11
Hungarian red wine									
Minimum	0.9916	6.3	1.40	0.158	0.019	0.53	—	0.33	0.06
Maximum	0.0974	11.1	3.43	0.272	0.051	1.05	—	1.41	0.28
Average	0.9952	9.0	2.54	0.215	0.038	0.67	—	0.79	0.15
Hungarian white wine									
Minimum	0.9907	5.4	1.45	0.126	0.014	0.45	—	0.41	—
Maximum	0.9993	10.0	3.50	0.504	0.068	1.01	0.78	1.22	—
Average	0.9955	8.0	2.33	0.204	0.034	0.69	—	0.77	—
Italian red wine									
Average	0.9940	10.5	3.44	0.290	0.032	0.52	0.44	1.45	—
Barletta	—	11.7	3.99	0.340	0.033	0.36	0.65	1.40	—
"	0.9955	10.3	3.10	0.290	0.031	0.60	0.50	0.85	—
"	0.9960	8.8	3.46	0.326	0.030	0.63	0.30	0.70	—
Brindisi	—	11.2	3.83	0.280	0.034	0.55	0.37	0.90	—
Chianti	0.9960	8.2	2.36	0.234	—	0.70	0.18	—	0.32
Sicilian wine									
Average	1.0094	12.7	7.55	0.380	—	0.63	8.41	—	0.16
Spanish red wine									
Average	—	12.1	3.53	0.610	0.027	0.49	0.38	1.09	0.22
Spanish (Alicante) sweet wine									
Average	1.0233	12.7	9.69	0.740	0.039	0.59	6.55	0.63	0.20

The Determination of Alcohol.—The amount of alcohol (by volume) may be determined by distilling 200 c.c., previously neutralized with a few drops of alkaline solution, and to which a few pieces of pumice stone have been added, and after collecting 150 c.c., making the distillate up to 200 c.c. and calculating the alcohol from the specific gravity of this (see table, p. 275). If the percentage by weight be required it is calculated by weighing the sample, and the distillate, when the amount of alcohol may be calculated as follows :—

Per cent by weight in the sample = $\frac{\text{weight of distillate}}{\text{weight of sample}} \times \text{per cent of alcohol in distillate.}$

It must be remembered that all alcohol determinations based on specific gravities are slightly erroneous in so far as esters or other secondary constituents are present.

Tabarie's method, which gives results close to the truth, is to evaporate the wine until all the alcohol is driven off and make up with water to the original volume. Then

$\frac{\text{Specific gravity of original sample}}{\text{Specific gravity of the "extract"}}$ = specific gravity of the alcohol present ; that is, the specific gravity of the sample minus its solid extract, from which the alcohol is at once calculated from the tables.

Alcohol in wines may also be determined with approximate accuracy by the use of the vaporimeter. This determination depends on the fact that the vapour tension at given temperatures of mixtures of alcohol and water can be measured by reference to the height of a column of mercury which is supported by the vapour pressure. In Geissler's vaporimeter, which is illustrated, the bulb A, quite dry, is filled up to the mark with mercury and then filled completely by the addition of the sample. The limb carrying the glass tube B and the scale, which has been experimentally determined, is fitted into the bulb, which the tube is ground to fit, and the bulb with the attached limb turned upside down, the wine thus rising to the closed end of the bulb ; the water jacket is now put into position and the water bath heated. As the temperature rises the alcohol becomes partially vaporized

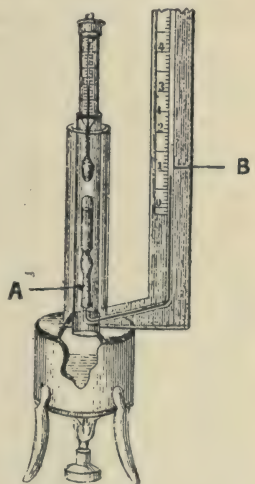


FIG. 35.—Vaporimeter.

and the mercury rises in the tube B. When the thermometer shows a constant temperature of 99.5° to 100°, and the mercury becomes steady, the amount is read off on the scale. Most vaporimeters are graduated for both volume and weight percentage. It is obvious that the weight percentage depends on the specific gravity of the wine—so that if this is materially different from unity, the observed result for alcohol by weight should be divided by the specific gravity.

Fixed Residue.—The determination of the fixed residue of wine presents several difficulties, owing to dehydration of sugars at elevated temperatures, and the fact that glycerine is present in small quantities. The following details should be observed, when the most accurate results possible are obtained. If the amount of extractive is less than 4 per cent a direct determination should be made. So much of the sample as will not leave more than 1.5 grms. of residue is evaporated for six hours on the water bath, and then transferred to a water oven for two hours, cooled in a desiccator and rapidly weighed. The evaporation should be done in a wide flat-bottomed dish. If the amount of residue be more than 4 per cent, a considerable proportion is generally sugar. The results of a direct determination are then inaccurate, and the amount of fixed residue may be calculated by determining the specific gravity of the liquid obtained by evaporating the alcohol from the sample and making up to the original bulk with water. On the assumption that a 10 per cent aqueous solution of wine solids has a specific gravity 1.0386 (which is not strictly correct, but is approximately so), the amount of fixed residue may be calculated from the specific gravity of the de-alcoholized sample by the table on following pages which is due to Windisch.

Mineral Matter.—The dry or nearly dry residue from the evaporation of 5 c.c. to 50 c.c. of the wine is ignited to whiteness and weighed.

Sugar and Polarization.—The polarization values of wine may often yield very useful results. The best methods are those laid down by the German official processes for wine analysis ("Centralblatt f. d. Deutsche Reich," 1896, No. 27) which are substantially as follows: An instrument of the Schmidt and Haensch type should be used and the results expressed in degrees of that instrument (or $\times 0.3468$ as angular rotation) on 200 mm. of the wine classified as described under sugar solutions. The following inferences are to be drawn:—

1. The wine is optically inactive. Either no sugar or other rotatory body is present in the wine, or there is a mixture of dextro- and lævo-rotatory sugars present. If after inversion by acid (see under sugars) the wine becomes lævo-rotatory cane sugar is almost certainly present. If the alcohol is driven off and the wine made up to its original volume, and the liquid is allowed to ferment by the addition of 2 grms. of air-dried yeast, and if it should then be dextro-rotatory, the indication is that lævo-rotatory sugar and commercial glucose, containing dextro-rotatory unfermentable substances were present. If no change is produced by inversion or fermentation, cane sugar, levulose and commercial glucose are absent.

2. The wine is dextro-rotatory. Cane sugar and/or commercial glucose are present. If after inversion it becomes lævo-rotatory, cane sugar was present. If after inversion it is dextro-rotatory to the extent of over 2.25° it is practically certain that this is due to the presence of unfermentable constituents of commercial glucose. If, after inversion, it is dextro-rotatory to the extent of from $+1^\circ$ to $+2.25^\circ$ it is treated as follows: 210 c.c. of the wine are evaporated to 70 c.c., made up with water to the original volume and fermented with 2

Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.
	Per cent		Per cent		Per cent		Per cent
1-0000	0-00	1-0050	1-29	1-0100	2-58	1-0150	3-87
1	0-03	1	1-32	1	2-61	1	3-90
2	0-05	2	1-34	2	2-63	2	3-93
3	0-08	3	1-37	3	2-66	3	3-95
4	0-10	4	1-39	4	2-69	4	3-98
5	0-13	5	1-42	5	2-71	5	4-00
6	0-15	6	1-45	6	2-74	6	4-03
7	0-18	7	1-47	7	2-76	7	4-06
8	0-20	8	1-50	8	2-79	8	4-08
9	0-23	9	1-52	9	2-82	9	4-11
1-0010	0-26	1-0060	1-55	1-0110	2-84	1-0160	4-13
1	0-28	1	1-57	1	2-87	1	4-16
2	0-31	2	1-60	2	2-89	2	4-19
3	0-34	3	1-63	3	2-92	3	4-21
4	0-36	4	1-65	4	2-94	4	4-24
5	0-39	5	1-68	5	2-97	5	4-26
6	0-41	6	1-70	6	3-00	6	4-29
7	0-44	7	1-73	7	3-02	7	4-31
8	0-46	8	1-76	8	3-05	8	4-34
9	0-49	9	1-78	9	3-07	9	4-37
1-0020	0-52	1-0070	1-81	1-0120	3-10	1-0170	4-39
1	0-54	1	1-83	1	3-12	1	4-42
2	0-57	2	1-86	2	3-15	2	4-44
3	0-59	3	1-88	3	3-18	3	4-47
4	0-62	4	1-91	4	3-20	4	4-50
5	0-64	5	1-94	5	3-23	5	4-52
6	0-67	6	1-96	6	3-26	6	4-55
7	0-69	7	1-99	7	3-28	7	4-57
8	0-72	8	2-01	8	3-31	8	4-60
9	0-75	9	2-04	9	3-33	9	4-63
1-0030	0-77	1-0080	2-07	1-0130	3-36	1-0180	4-65
1	0-80	1	2-09	1	3-38	1	4-68
2	0-82	2	2-12	2	3-41	2	4-70
3	0-85	3	2-14	3	3-43	3	4-73
4	0-87	4	2-17	4	3-46	4	4-75
5	0-90	5	2-19	5	3-49	5	4-78
6	0-93	6	2-22	6	3-51	6	4-81
7	0-95	7	2-25	7	3-54	7	4-83
8	0-98	8	2-27	8	3-56	8	4-86
9	1-00	9	2-30	9	3-59	9	4-88
1-0040	1-03	1-0090	2-32	1-0140	3-62	1-0190	4-91
1	1-05	1	2-35	1	3-64	1	4-94
2	1-08	2	2-38	2	3-67	2	4-96
3	1-11	3	2-40	3	3-69	3	4-99
4	1-13	4	2-43	4	3-72	4	5-01
5	1-16	5	2-45	5	3-75	5	5-04
6	1-18	6	2-48	6	3-77	6	5-06
7	1-21	7	2-50	7	3-80	7	4-09
8	1-24	8	2-53	8	3-82	8	5-11
9	1-26	9	2-56	9	3-85	9	5-14

Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.
	Per cent		Per cent		Per cent		Per cent
1-0200	5-17	1-0250	6-46	1-0300	7-76	1-0350	9-05
1	5-19	1	6-49	1	7-78	1	9-08
2	5-22	2	6-51	2	7-81	2	9-10
3	5-25	3	6-54	3	7-83	3	9-13
4	5-27	4	6-56	4	7-86	4	9-16
5	5-30	5	6-59	5	7-89	5	9-18
6	5-32	6	6-62	6	7-91	6	9-21
7	5-35	7	6-64	7	7-94	7	9-23
8	5-38	8	6-67	8	7-97	8	9-26
9	5-40	9	6-70	9	7-99	9	9-29
1-0210	5-43	1-0260	6-72	1-0310	8-02	1-0360	9-31
1	5-45	1	6-75	1	8-04	1	9-34
2	5-48	2	6-77	2	8-07	2	9-36
3	5-51	3	6-80	3	8-09	3	9-39
4	5-53	4	6-82	4	8-12	4	9-42
5	5-56	5	6-85	5	8-14	5	9-44
6	5-58	6	6-88	6	8-17	6	9-47
7	5-61	7	6-90	7	8-20	7	9-49
8	5-64	8	6-93	8	8-22	8	9-52
9	5-66	9	6-95	9	8-25	9	9-55
1-0220	5-69	1-0270	6-98	1-0320	8-27	1-0370	9-57
1	5-71	1	7-01	1	8-30	1	9-60
2	5-74	2	7-03	2	8-33	2	9-62
3	5-77	3	7-06	3	8-35	3	9-65
4	5-79	4	7-08	4	8-38	4	9-68
5	5-82	5	7-11	5	8-40	5	9-70
6	5-84	6	7-13	6	8-43	6	9-73
7	5-87	7	7-16	7	8-46	7	9-75
8	5-89	8	7-19	8	8-48	8	9-78
9	5-92	9	7-21	9	8-51	9	9-80
1-0230	5-94	1-0280	7-24	1-0330	8-53	1-0380	9-83
1	5-97	1	7-26	1	8-56	1	9-86
2	6-00	2	7-29	2	8-59	2	9-88
3	6-02	3	7-32	3	8-61	3	9-91
4	6-05	4	7-34	4	8-64	4	9-93
5	6-07	5	7-37	5	8-66	5	9-96
6	6-10	6	7-39	6	8-69	6	9-99
7	6-12	7	7-42	7	8-72	7	10-01
8	6-15	8	7-45	8	8-74	8	10-04
9	6-18	9	7-47	9	8-77	9	10-06
1-0240	6-20	1-0290	7-50	1-0340	8-79	1-0390	10-09
1	6-23	1	7-52	1	8-82	1	10-11
2	6-25	2	7-55	2	8-85	2	10-14
3	6-28	3	7-58	3	8-87	3	10-17
4	6-31	4	7-60	4	8-90	4	10-19
5	6-33	5	7-63	5	8-92	5	10-22
6	6-36	6	7-65	6	8-95	6	10-25
7	6-38	7	7-68	7	8-97	7	10-27
8	6-41	8	7-70	8	9-00	8	10-30
9	6-44	9	7-73	9	9-03	9	10-32

Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.
	Per cent		Per cent		Per cent		Per cent
1-0400	10-35	1-0450	11-65	1-0500	12-95	1-0550	14-25
1	10-37	1	11-68	1	12-97	1	14-28
2	10-40	2	11-70	2	13-00	2	14-30
3	10-43	3	11-73	3	13-03	3	14-33
4	10-45	4	11-75	4	13-05	4	14-35
5	10-48	5	11-78	5	13-08	5	14-38
6	10-51	6	11-81	6	13-10	6	14-41
7	10-53	7	11-83	7	13-13	7	14-43
8	10-56	8	11-86	8	13-16	8	14-46
9	10-58	9	11-88	9	13-18	9	14-48
1-0410	10-61	1-0460	11-91	1-0510	13-21	1-0560	14-51
1	10-63	1	11-94	1	13-23	1	14-54
2	10-66	2	11-96	2	13-26	2	14-56
3	10-69	3	11-99	3	13-29	3	14-59
4	10-71	4	12-01	4	13-31	4	14-61
5	10-74	5	12-04	5	13-34	5	14-64
6	10-76	6	12-06	6	13-36	6	14-67
7	10-79	7	12-09	7	13-39	7	14-69
8	10-82	8	12-12	8	13-42	8	14-72
9	10-84	9	12-14	9	13-44	9	14-74
1-0420	10-87	1-0470	12-17	1-0520	13-47	1-0570	14-77
1	10-90	1	12-19	1	13-49	1	14-80
2	10-92	2	12-22	2	13-52	2	14-82
3	10-95	3	12-25	3	13-55	3	14-85
4	10-97	4	12-27	4	13-57	4	14-87
5	11-00	5	12-30	5	13-60	5	14-90
6	11-03	6	12-32	6	13-62	6	14-93
7	11-05	7	12-35	7	13-65	7	14-95
8	11-08	8	12-38	8	13-68	8	14-98
9	11-10	9	12-40	9	13-70	9	15-00
1-0430	11-13	1-0480	12-43	1-0530	13-73	1-0580	15-03
1	11-15	1	12-45	1	13-75	1	15-06
2	11-18	2	12-48	2	13-78	2	15-08
3	11-21	3	12-51	3	13-81	3	15-11
4	11-23	4	12-53	4	13-83	4	15-14
5	11-26	5	12-56	5	13-86	5	15-16
6	11-28	6	12-58	6	13-89	6	15-19
7	11-31	7	12-61	7	13-91	7	15-22
8	11-34	8	12-64	8	13-94	8	15-24
9	11-36	9	12-66	9	13-96	9	15-27
1-0440	11-39	1-0490	12-69	1-0540	13-99	1-0590	15-30
1	11-42	1	12-71	1	14-01	1	15-32
2	11-44	2	12-74	2	14-04	2	15-35
3	11-47	3	12-77	3	14-07	3	15-37
4	11-49	4	12-79	4	14-09	4	15-40
5	11-52	5	12-82	5	14-12	5	15-42
6	11-55	6	12-84	6	14-14	6	15-45
7	11-57	7	12-87	7	14-17	7	15-48
8	11-60	8	12-90	8	14-20	8	15-50
9	11-62	9	12-92	9	14-22	9	15-53

Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.
	Per cent		Per cent		Per cent		Per cent
1-0600	15-55	1-0650	16-86	1-0700	18-16	1-0750	19-47
1	15-58	1	16-88	1	18-19	1	19-50
2	15-61	2	16-91	2	18-22	2	19-52
3	15-63	3	16-94	3	18-24	3	19-55
4	15-66	4	16-96	4	18-27	4	19-58
5	15-68	5	16-99	5	18-30	5	19-60
6	15-71	6	17-01	6	18-32	6	19-63
7	15-74	7	17-04	7	18-35	7	19-65
8	15-76	8	17-07	8	18-37	8	19-68
9	15-79	9	17-09	9	18-40	9	19-71
1-0610	15-81	1-0660	17-12	1-0710	18-43	1-0760	19-73
1	15-84	1	17-14	1	18-45	1	19-76
2	15-87	2	17-17	2	18-48	2	19-79
3	15-89	3	17-20	3	18-50	3	19-81
4	15-92	4	17-22	4	18-53	4	19-84
5	15-94	5	17-25	5	18-56	5	19-86
6	15-97	6	17-27	6	18-58	6	19-89
7	16-00	7	17-30	7	18-61	7	19-92
8	16-02	8	17-33	8	18-63	8	19-94
9	16-05	9	17-35	9	18-66	9	19-97
1-0620	16-07	1-0670	17-38	1-0720	18-69	1-0770	20-00
1	16-10	1	17-41	1	18-71	1	20-02
2	16-13	2	17-43	2	18-74	2	20-05
3	16-15	3	17-46	3	18-76	3	20-07
4	16-18	4	17-48	4	18-79	4	20-10
5	16-21	5	17-51	5	18-82	5	20-12
6	16-23	6	17-54	6	18-84	6	20-15
7	16-26	7	17-56	7	18-87	7	20-18
8	16-28	8	17-59	8	18-90	8	20-20
9	16-31	9	17-62	9	18-92	9	20-23
1-0630	16-33	1-0680	17-64	1-0730	18-95	1-0780	20-26
1	16-36	1	17-67	1	18-97	1	20-28
2	16-39	2	17-69	2	19-00	2	20-31
3	16-41	3	17-72	3	19-03	3	20-34
4	16-44	4	17-75	4	19-05	4	20-36
5	16-47	5	17-77	5	19-08	5	20-39
6	16-49	6	17-80	6	19-10	6	20-41
7	16-52	7	17-83	7	19-13	7	20-44
8	16-54	8	17-85	8	19-16	8	20-47
9	16-57	9	17-88	9	19-18	9	20-49
1-0640	16-60	1-0690	17-90	1-0740	19-21	1-0790	20-52
1	16-62	1	17-93	1	19-23	1	20-55
2	16-65	2	17-95	2	19-26	2	20-57
3	16-68	3	17-98	3	19-29	3	20-60
4	16-70	4	18-01	4	19-31	4	20-62
5	16-73	5	18-03	5	19-34	5	20-65
6	16-75	6	18-06	6	19-37	6	20-68
7	16-78	7	18-08	7	19-39	7	20-70
8	16-80	8	18-11	8	19-42	8	20-73
9	16-83	9	18-14	9	19-44	9	20-75

Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.	Sp. Gr.	Residue.
	Per cent		Per cent		Per cent		Per cent
1-0800	20-78	1-0840	21-83	1-0880	22-88	1-0920	23-93
1	20-81	1	21-86	1	22-91	1	23-96
2	20-83	2	21-88	2	22-93	2	23-99
3	20-86	3	21-91	3	22-96	3	24-01
4	20-89	4	21-94	4	22-99	4	24-04
5	20-91	5	21-96	5	23-01	5	24-07
6	20-94	6	21-99	6	23-04	6	24-09
7	20-96	7	22-02	7	23-07	7	24-12
8	20-99	8	22-04	8	23-09	8	24-14
9	21-02	9	22-07	9	23-12	9	24-17
1-0810	21-04	1-0850	22-09	1-0890	23-14	1-0930	24-20
1	21-07	1	22-12	1	23-17	1	24-22
2	21-10	2	22-15	2	23-20	2	24-25
3	21-12	3	22-17	3	23-22	3	24-27
4	21-15	4	22-20	4	23-25	4	24-30
5	21-17	5	22-22	5	23-28	5	24-33
6	21-20	6	22-25	6	23-30	6	24-35
7	21-23	7	22-28	7	23-33	7	24-38
8	21-25	8	22-30	8	23-35	8	24-41
9	21-28	9	22-33	9	23-38	9	24-43
1-0820	21-31	1-0860	22-36	1-0900	23-41	1-0940	24-46
1	21-33	1	22-38	1	23-43	1	24-49
2	21-36	2	22-41	2	23-46	2	24-51
3	21-38	3	22-43	3	23-49	3	24-54
4	21-41	4	22-46	4	23-51	4	24-57
5	21-44	5	22-49	5	23-54	5	24-59
6	21-46	6	22-51	6	23-57	6	24-62
7	21-49	7	22-54	7	23-59	7	24-64
8	21-52	8	22-57	8	23-62	8	24-67
9	21-54	9	22-59	9	23-65	9	24-70
1-0830	21-57	1-0870	22-62	1-0910	23-67	1-0950	24-72
1	21-59	1	22-65	1	23-70	1	24-75
2	21-62	2	22-67	2	23-72	2	24-78
3	21-65	3	22-70	3	23-75	3	24-80
4	21-67	4	22-72	4	23-77	4	24-83
5	21-70	5	22-75	5	23-80	5	24-85
6	21-73	6	22-78	6	23-83	6	24-88
7	21-75	7	22-80	7	23-85	7	24-91
8	21-78	8	22-83	8	23-88	8	24-93
9	21-80	9	22-86	9	23-91	9	24-96

grms. of air-dried yeast. The liquid is then evaporated with a little sand and a few drops of 20 per cent solution of potassium acetate, to a thin syrup; 200 c.c. of 90 per cent alcohol are then added, with constant stirring. The liquid is filtered and the whole evaporated to about 5 c.c. The residue is mixed with bone black, filtered, and the filter washed with water until the filtrate measures 30 c.c. If the filtrate has a dextro-rotation of more than 1.5° the unfermentable constituents of commercial glucose were present.

3. The wine is lævo-rotatory. It must contain unfermented lævo-

rotatory sugar which may be natural and/or inverted cane sugar. Some dextro-rotatory sugar may, of course, also be present. If after fermentation as above described the lævo-rotation is at least -3° , only lævo-rotatory sugar was present. If it now rotates to the right, lævo-rotatory sugar and commercial glucose were present. If the lævo-rotation is increased by inversion, both lævo-rotatory sugar and unchanged cane sugar are present.

The German official processes are substantially as follows:—

White Wines.—Sixty c.c. are neutralized with alkali, evaporated to one-third their volume and made up again with water. Three c.c. of basic acetate of lead solution (10 per cent solution) are added and the liquid filtered; 31.5 c.c. of the filtrate are treated with 1.5 c.c. of a saturated solution of Na_2CO_3 and filtered. The liquid is now diluted in the proportion of 10 to 11 so that for a 200 mm. reading a 220 tube must be used.

Red Wines.—To the 60 c.c. of de-alcoholized wine 6 c.c. of the lead subacetate solution are added, and to 33 c.c. of the filtrate 3 c.c. of a saturated solution of Na_2CO_3 are added. The filtered liquid now represents the wine diluted from 5 to 6, so that if the reading be taken in a 200 mm. tube it must be multiplied by 1.2.

A little animal charcoal may be used, if decolorization is not complete by the use of lead subacetate.

For the inversion of the wine to correspond with the values given above, the following process must be used:—

One hundred c.c. are neutralized, evaporated to one-third, made up to original volume and decolorized with 2 c.c. of lead subacetate solution, and 8 c.c. of water added. To 55 c.c. of the filtered solution 0.5 c.c. of saturated Na_2CO_3 solution is added, and 4.5 c.c. of water, and the whole filtered. The dilution is now 5 to 6, so that the 200 mm. direct reading is multiplied by 1.2. Thirty-three c.c. of the filtrate from the lead subacetate is now inverted by adding 3 c.c. of strong HCl , and heating in ten minutes to 70°C . It is then quickly cooled, filtered, and the rotation is a 200 mm. multiplied by 1.2. This gives the true value after inversion.

For readings after fermentation, except in the special case above described, 50 c.c. are de-alcoholized, made up to original volume and kept at 30° for sixty hours with washed yeast. A few drops of a solution of acid mercuric nitrate followed by a few drops of solution of subacetate of lead, are then added and finally a little sodium carbonate solution. The whole is then filtered, made up to 100 c.c. and the reading taken in a 200 mm. tube. The dilution is 1 to 2, so that the reading is multiplied by 2.

For the determination of the reducing sugars, the French official method is the volumetric process. One hundred c.c. of wine are neutralized by sodium bicarbonate, and a few c.c. of 10 per cent subacetate of lead solution added, excess being avoided. The volume is made up to 110 c.c., the whole well shaken and filtered. If the liquid is still coloured, it is shaken with some animal charcoal, and again filtered. Five c.c. of Fehling's solution (= 0.025 gm. of glucose) are used for the titration. If the amount of wine necessary to de-

colorize the Fehling's solution is less than 5 c.c., it is diluted sufficiently for the amount finally used to lie between 5 c.c. and 10 c.c. In calculating, it is to be remembered that 11 c.c. of the liquid are equivalent to 10 c.c. of the wine.

In Germany the official method is the gravimetric process. If 25 c.c. of the above filtrate be used, and the results multiplied by 1.1, the resulting precipitate can be weighed as metallic copper after reduction by a stream of hydrogen. This is the official German process, and the results are calculated from Weiss' tables, which are given on pp. 324-5, showing the amount of invert sugar present.

If the precipitate be weighed as CuO , then the amounts of sugar in the above table must be multiplied by the factor 0.8.

For the determination of cane sugar 50 c.c. of the filtrate used for the determination of reducing sugars are treated with 5 c.c. of 5 per cent HCl and the liquid heated on a water bath for twenty minutes. The liquid is exactly neutralized with Na_2CO_3 , evaporated slightly, rendered faintly alkaline with Na_2CO_3 , and filtered, any residue being washed with water until the filtrate measures 50 c.c. The amount of reducing sugar is now determined by means of Fehling's solution as before, 11 c.c. of the solution being equivalent to 10 c.c. of the original wine. Further, as 95 parts of cane sugar yield 100 parts of invert sugar, the real amount of cane sugar present is given by the formula $x = 0.95(b - a)$ where b is the amount of invert sugar formed after, and a the amount before, inversion. The gravimetric process is preferable.

Acidity.—Twenty-five c.c. of the wine are heated until boiling just commences, and titrated with $\frac{N}{4}$ potassium hydroxide. Litmus paper should be used as an indicator, and in order to ensure accurate results the alkali should be standardized against a solution of tartaric acid, using the same indicator. If standardized exactly, each c.c. of the $\frac{N}{4}$ alkali is equivalent to 18.75 mg. of tartaric acid (assuming that the whole of the free acids are tartaric, which is not strictly true). The official French standards are calculated to grams of sulphuric acid per litre, and $\frac{N}{20}$ sodium hydroxide solution is used for the titration, using phenol-phthalein as indicator. This is somewhat difficult in the case of red wines, unless used in the form of spots on a white tile.

To separate the fixed and volatile free acids, the following apparatus, which is used officially in Germany will be found the most useful. Fifty c.c. of the wine are placed in the flask B, which holds 200 c.c., and a little tannic acid added in order to prevent foaming.

At first the connexion between the distilling flask and the steam-generating flask A, is interrupted by a clip on the india-rubber portion of the connexion. The wine is distilled until reduced to half its volume, the distillate being collected in the flask C. Steam is then turned on, the flame below B being lowered, and 200 c.c. is collected.

Cu.	Sugar.	Cu.	Sugar.	Cu.	Sugar.	Cu	Sugar.
Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
0-010	0-0061	0-063	0-0323	0-116	0-0607	0-169	0-0892
0-011	0-0066	0-064	0-0328	0-117	0-0612	0-170	0-0897
0-012	0-0071	0-065	0-0333	0-118	0-0617	0-171	0-0903
0-013	0-0076	0-066	0-0338	0-119	0-0623	0-172	0-0908
0-014	0-0081	0-067	0-0343	0-120	0-0628	0-173	0-0914
0-015	0-0086	0-068	0-0348	0-121	0-0633	0-174	0-0919
0-016	0-0090	0-069	0-0353	0-122	0-0639	0-175	0-0924
0-017	0-0095	0-070	0-0358	0-123	0-0644	0-176	0-0930
0-018	0-0100	0-071	0-0363	0-124	0-0649	0-177	0-0935
0-019	0-0105	0-072	0-0368	0-125	0-0655	0-178	0-0941
0-020	0-0110	0-073	0-0373	0-126	0-0660	0-179	0-0946
0-021	0-0115	0-074	0-0378	0-127	0-0665	0-180	0-0952
0-022	0-0120	0-075	0-0383	0-128	0-0671	0-181	0-0957
0-023	0-0125	0-076	0-0388	0-129	0-0676	0-182	0-0962
0-024	0-0130	0-077	0-0393	0-130	0-0681	0-183	0-0968
0-025	0-0135	0-078	0-0398	0-131	0-0687	0-184	0-0973
0-026	0-0140	0-079	0-0403	0-132	0-0692	0-185	0-0978
0-027	0-0145	0-080	0-0408	0-133	0-0697	0-186	0-0984
0-028	0-0150	0-081	0-0413	0-134	0-0703	0-187	0-0990
0-029	0-0155	0-082	0-0418	0-135	0-0708	0-188	0-0995
0-030	0-0160	0-083	0-0423	0-136	0-0713	0-189	0-1001
0-031	0-0165	0-084	0-0428	0-137	0-0719	0-190	0-1006
0-032	0-0170	0-085	0-0434	0-138	0-0724	0-191	0-1012
0-033	0-0175	0-086	0-0439	0-139	0-0729	0-192	0-1017
0-034	0-0180	0-087	0-0444	0-140	0-0735	0-193	0-1023
0-035	0-0185	0-088	0-0449	0-141	0-0740	0-194	0-1029
0-036	0-0189	0-089	0-0454	0-142	0-0745	0-195	0-1034
0-037	0-0194	0-090	0-0469	0-143	0-0751	0-196	0-1040
0-038	0-0199	0-091	0-0474	0-144	0-0756	0-197	0-1046
0-039	0-0204	0-092	0-0479	0-145	0-0761	0-198	0-1051
0-040	0-0209	0-093	0-0484	0-146	0-0767	0-199	0-1057
0-041	0-0214	0-094	0-0489	0-147	0-0772	0-200	0-1063
0-042	0-0219	0-095	0-0495	0-148	0-0778	0-201	0-1068
0-043	0-0224	0-096	0-0500	0-149	0-0783	0-202	0-1074
0-044	0-0229	0-097	0-0505	0-150	0-0789	0-203	0-1079
0-045	0-0234	0-098	0-0511	0-151	0-0794	0-204	0-1085
0-046	0-0239	0-099	0-0516	0-152	0-0800	0-205	0-1091
0-047	0-0244	0-100	0-0521	0-153	0-0805	0-206	0-1096
0-048	0-0249	0-101	0-0527	0-154	0-0810	0-207	0-1102
0-049	0-0254	0-102	0-0532	0-155	0-0816	0-208	0-1108
0-050	0-0259	0-103	0-0537	0-156	0-0821	0-209	0-1113
0-051	0-0264	0-104	0-0543	0-157	0-0827	0-210	0-1119
0-052	0-0269	0-105	0-0548	0-158	0-0832	0-211	0-1125
0-053	0-0274	0-106	0-0553	0-159	0-0838	0-212	0-1130
0-054	0-0279	0-107	0-0559	0-160	0-0843	0-213	0-1136
0-055	0-0284	0-108	0-0565	0-161	0-0848	0-214	0-1142
0-056	0-0288	0-109	0-0569	0-162	0-0854	0-215	0-1147
0-057	0-0293	0-110	0-0575	0-163	0-0859	0-216	0-1153
0-058	0-0298	0-111	0-0580	0-164	0-0865	0-217	0-1158
0-059	0-0303	0-112	0-0585	0-165	0-0870	0-218	0-1164
0-060	0-0308	0-113	0-0591	0-166	0-0876	0-219	0-1170
0-061	0-0313	0-114	0-0596	0-167	0-0881	0-220	0-1175
0-062	0-0318	0-115	0-0601	0-168	0-0886	0-221	0-1181

Cu.	Sugar.	Cu.	Sugar.	Cu.	Sugar.	Cu.	Sugar.
Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
0-222	0-1187	0-278	0-1507	0-334	0-1841	0-390	0-2187
0-223	0-1192	0-279	0-1513	0-335	0-1847	0-391	0-2193
0-224	0-1198	0-280	0-1519	0-336	0-1854	0-392	0-2199
0-225	0-1204	0-281	0-1525	0-337	0-1860	0-393	0-2205
0-226	0-1209	0-282	0-1531	0-338	0-1866	0-394	0-2212
0-227	0-1215	0-283	0-1537	0-339	0-1872	0-395	0-2218
0-228	0-1221	0-284	0-1543	0-340	0-1878	0-396	0-2224
0-229	0-1226	0-285	0-1549	0-341	0-1884	0-397	0-2231
0-230	0-1232	0-286	0-1555	0-342	0-1890	0-398	0-2237
0-231	0-1238	0-287	0-1561	0-343	0-1896	0-399	0-2243
0-232	0-1243	0-288	0-1567	0-344	0-1902	0-400	0-2249
0-233	0-1249	0-289	0-1572	0-345	0-1908	0-401	0-2255
0-234	0-1255	0-290	0-1578	0-346	0-1914	0-402	0-2264
0-235	0-1260	0-291	0-1584	0-347	0-1920	0-403	0-2271
0-236	0-1266	0-292	0-1590	0-348	0-1926	0-404	0-2278
0-237	0-1272	0-293	0-1596	0-349	0-1932	0-405	0-2286
0-238	0-1278	0-294	0-1602	0-350	0-1938	0-406	0-2293
0-239	0-1283	0-295	0-1608	0-351	0-1944	0-407	0-2300
0-240	0-1289	0-296	0-1614	0-352	0-1950	0-408	0-2307
0-241	0-1295	0-297	0-1620	0-353	0-1956	0-409	0-2314
0-242	0-1300	0-298	0-1626	0-354	0-1962	0-410	0-2321
0-243	0-1306	0-299	0-1632	0-355	0-1968	0-411	0-2328
0-244	0-1312	0-300	0-1638	0-356	0-1974	0-412	0-2335
0-245	0-1318	0-301	0-1644	0-357	0-1980	0-413	0-2343
0-246	0-1323	0-302	0-1650	0-358	0-1986	0-414	0-2350
0-247	0-1329	0-303	0-1656	0-359	0-1992	0-415	0-2357
0-248	0-1335	0-304	0-1662	0-360	0-1998	0-416	0-2364
0-249	0-1341	0-305	0-1668	0-361	0-2004	0-417	0-2371
0-250	0-1346	0-306	0-1673	0-362	0-2011	0-418	0-2378
0-251	0-1352	0-307	0-1679	0-363	0-2017	0-419	0-2385
0-252	0-1358	0-308	0-1685	0-364	0-2023	0-420	0-2392
0-253	0-1363	0-309	0-1691	0-365	0-2030	0-421	0-2399
0-254	0-1369	0-310	0-1697	0-366	0-2036	0-422	0-2406
0-255	0-1375	0-311	0-1703	0-367	0-2042	0-423	0-2413
0-256	0-1381	0-312	0-1709	0-368	0-2048	0-424	0-2420
0-257	0-1386	0-313	0-1715	0-369	0-2055	0-425	0-2427
0-258	0-1392	0-314	0-1721	0-370	0-2061	0-426	0-2434
0-259	0-1398	0-315	0-1727	0-371	0-2067	0-427	0-2441
0-260	0-1404	0-316	0-1733	0-372	0-2073	0-428	0-2449
0-261	0-1409	0-317	0-1739	0-373	0-2080	0-429	0-2456
0-262	0-1415	0-318	0-1745	0-374	0-2086	0-430	0-2463
0-263	0-1421	0-319	0-1751	0-375	0-2092		
0-264	0-1427	0-320	0-1756	0-376	0-2099		
0-265	0-1432	0-321	0-1762	0-377	0-2105		
0-266	0-1438	0-322	0-1768	0-378	0-2111		
0-267	0-1444	0-323	0-1774	0-379	0-2117		
0-268	0-1449	0-324	0-1780	0-380	0-2124		
0-269	0-1455	0-325	0-1786	0-381	0-2130		
0-270	0-1461	0-326	0-1792	0-382	0-2136		
0-271	0-1467	0-327	0-1798	0-383	0-2143		
0-272	0-1472	0-328	0-1804	0-384	0-2149		
0-273	0-1478	0-329	0-1810	0-385	0-2155		
0-274	0-1484	0-330	0-1816	0-386	0-2161		
0-275	0-1490	0-331	0-1822	0-387	0-2168		
0-276	0-1495	0-332	0-1828	0-388	0-2174		
0-277	0-1501	0-333	0-1835	0-389	0-2180		

The distillate is titrated with standard alkali, and the results calculated to acetic acid. By deducting the amount of alkali used for the neutralization of the volatile acids for 100 c.c. of wine, from that used for the total acids, the remainder is calculated into tartaric acid and returned as fixed acids.



FIG. 36.—Apparatus for determining volatile acids in wine.

Glycerine.—Approximate results may be obtained by the use of the process devised by Trillat ("Comptes Rendus," 135, 903), which is as follows:—

Fifty c.c. of wine is evaporated in a small silver dish on the water bath at 70° C. to one third of its volume. Five grms. of animal charcoal are then added, intimately mixed with the residue, and evaporation continued to complete dryness. After cooling, this residue is mixed with 5 grms. of quicklime. The powder thus obtained is transferred to a flask and agitated for five minutes with 30 c.c. of pure dry acetic ether. The liquid is decanted and filtered, and the powder extracted twice more with the same quantity of solvent. The acetic ether is then evaporated, in small quantities at a time, in a tared capsule on the water bath, then dried to constant weight at 60° C. and weighed. It may then be ignited and the ash weighed, this weight being deducted from that of the glycerin; but, as a rule, the amount of ash is so small that it may be disregarded.

The German official method is tedious but gives fairly exact results except in the case of plastered wines when they are too high. The following are the details of this method:—

(1) Wines containing less than 2 per cent of sugar: 100 c.c. are evaporated down to 15 c.c. on a water bath, and 1 grm. of fine sand added. Two c.c. of 40 per cent emulsion of lime are added for each grm. of fixed residue present, and evaporation continued. When the water is nearly driven off, 5 c.c. of 96 per cent alcohol are added. The particles adhering to the sides of the dish are loosened with a glass rod and rubbed into a cream with a little more alcohol. The mixture is heated on the water bath with constant stirring until it begins to boil, when the liquid is decanted into a 100 c.c. flask. The residue in the dish is extracted with five or six portions of 10 c.c. of 96 per cent

alcohol, each portion being decanted into the flask, which is then made up to 100 c.c. with alcohol. After filtration, 90 c.c. of the filtrate are evaporated in a porcelain dish on the water bath, which is only allowed to boil very gently. When the alcohol is driven off the residue in dish is washed out with three successive portions of 5 c.c. each of absolute alcohol, which are transferred to a graduated cylinder, and made up to exactly 15 c.c. with absolute alcohol. Three successive portions of 7.5 c.c. of absolute ether are added to the contents of the cylinder which are well shaken after each addition. When the solution is quite clear it is transferred to a tared glass dish, and the cylinder washed out with a mixture of 2 c.c. of alcohol and 3 of ether. The alcohol-ether is evaporated in a warm water bath—care being taken that the solvent does not actually boil—the residue is dried in a water oven for one hour, cooled in a desiccator and rapidly weighed.

Wines containing more than 2 per cent of sugar: 50 c.c. are warmed in a large flask on the water bath, and 1 grm. of fine sand, and milk of lime until the colour is quite pale, are added. On cooling 100 c.c. of 96 per cent alcohol are added, the precipitate is allowed to subside, and the liquid filtered, the precipitate and filter being washed with strong alcohol. The filtrate is then treated as in the former case.

Stierlin's method is to evaporate the liquid, without adding anything, to one-fifth or one-sixth of its volume. He then extracts with hot absolute alcohol, and estimates sugar, non-volatile acids, alkaloïds, bitter matters and glycerin in this alcoholic extract. Glycerin is estimated by freeing a given quantity from alcohol by evaporation, then again evaporating to dryness with a slight excess of caustic lime. It is then extracted with alcohol and ether (2 : 3), or alcohol and chloroform may be used.

Raynaud has stated that the processes used for the estimation of glycerin cannot always be depended upon, especially with plastered wines, when the results obtained are too high, since lime decomposes a large amount of sulphate of potash and hydrate of potash is formed, which is dissolved by glycerin when alcohol is present, and is of course weighed with it. He suggests the following process. Evaporate the liquid to one-fifth of its volume, and precipitate the potash by hydrofluosilicic acid. Then filter the liquid. Add baryta water to make slightly alkaline, also a small amount of sand, and evaporate to dryness in a vacuum: to extract the dry residue add a very large quantity of absolute alcohol and ether; as much as 300 c.c. for 250 c.c. of wine, can be used. This, however, is unnecessary with proper extracting apparatus, and 50 c.c. to 100 c.c. in a Soxhlet's apparatus will have just the same effect. When the alcohol and ether have evaporated the glycerin should stand for twenty-four hours in a vacuum over phosphoric anhydride; it is then put into a tube, a perfect vacuum formed, and at a temperature of 180° it will distil into the cool part of the tube.

A useful method of estimating glycerine is that of Parthiel, by distillation in a vacuum to separate the more volatile substances and oxidation of the glycerin to oxalic acid. He takes 50 c.c. of the liquid and adds a little calcium carbonate to neutralize it. It is

evaporated down to 15 c.c. and placed in a small retort which is enclosed in an air bath, the bottom of the bath being made of sheet iron, while the sides and top are made of asbestos card. A globular receiver is connected with the neck. The second opening of the receiver is connected with an inverted condenser, and then to a pump. The receiver is kept cool. The liquid is first distilled almost to dryness at ordinary pressure, the temperature being 120°C . Then it is cooled to 60°C . The pressure is reduced by means of a pump, the temperature being 80°C . and it is now distilled for one and a half hours: then the vacuum is broken, the retort cooled, 10 c.c. of water are added and distillation is continued at the ordinary pressure, the temperature in the bath being 120°C . The distillate is then diluted to 200 c.c., 8 grm. to 10 grms. of caustic soda are dissolved in it, and 5 per cent of potassium permanganate are added until there is an unmistakable blue-black colour. The whole should then be heated for an hour, SO_2 added to decolorize, 20 c.c. of acetic acid added, the whole heated to get rid of SO_2 and the oxalic acid precipitated by calcium chloride.

The iodide method proposed by Zeisel and Fanto for the estimation of glycerin in wine is quite trustworthy. In this method the wine is prepared for analysis by treating 100 c.c. of it with tannin and barium acetate, distilling off about 70 c.c. and diluting the residue to 100 c.c. Five c.c. of this solution are then distilled in a current of carbon dioxide after the addition of hydriodic acid. The isopropyl iodide formed by the action of the hydriodic acid on the free and combined glycerin distils over, and after being passed through a small wash bottle containing amorphous phosphorus suspended in water, is collected in an alcoholic solution of silver nitrate. The quantity of silver iodide produced corresponds with the amount of glycerin present.

The lime method, in the case of wines containing not more than 5 per cent of sugar, yields results which are somewhat lower than those obtained by the iodide method.

With sweet wines much lower results are obtained by the lime method than by the iodide method.

There are numerous other methods for the estimation of glycerin, but all of a more or less complicated character and none yielding strictly accurate results.

For these reference may be made to the original publications, as follows:—

Bordas and de Raczkowski (oxidation by chromic acid, "*Comptes Rendus*," 1896, 1021).

Bottinger (conversion into triacetin, "*Comptes Rendus*," 1897, 240).

Sulphates.—The significance of any excessive quantity of sulphates in a wine has reference to the practice known as plastering. A good deal has been said against the practice of adding a small amount of calcium sulphate to wines, but so long as but little is used, the facility with which the wine is clarified entirely outweighs any sentimental disadvantages attached to the process. The greater part of the cream of tartar present in the wine is converted into insoluble calcium tartrate, which mechanically carries down various impurities in the wine which

would otherwise require an exceedingly long time to settle down. The practice of plastering is general in the sherry district—indeed the author is informed by leading wine experts that it is a commercial necessity with this wine, but it is also resorted to to a lesser extent in other districts. There is always a small amount of potassium sulphate present in grape juice, and a small quantity results from the practice of sulphuring the casks, the SO_2 generated becoming oxidized to sulphuric acid. An unplastered wine will contain sulphates to the extent of 0.1 per cent calculated as potassium sulphate. Any excess over 0.2 is usually accepted as evidence of plastering; indeed, any excess over 0.1 per cent is nearly always due to plastering. Native wines in Germany and most wines in France or Switzerland are not allowed to be sold with over 0.2 per cent of sulphates, calculated as potassium sulphate. The sulphates are determined by evaporating 100 c.c. to about 30 c.c., and adding excess of hot solution of BaCl_2 in the usual manner, after acidification with hydrochloric acid, and weighing as BaSO_4 .

Sulphurous Acid.—The presence of sulphurous acid in wine may be due to traces being absorbed from sulphured casks, or it may be due to the addition of sulphurous acid or sulphites for the purpose of preserving the wine, a quite necessary precaution for certain types of wine. Sulphurous acid, when added to wine, appears to enter, to a very considerable extent, into combination with normal constituents of the wine, leaving a relatively small amount in the free state. In the determination of sulphurous acid it is customary to return the SO_2 as “free” and “total,” the total including this combined or “aldehyde” sulphurous acid. The combined sulphurous acid is regarded as almost innocuous, whilst objection is taken to more than traces of free acid. The usual official limits are 200 mg. of SO_2 per litre for the total, or 20 mg. to 30 mg. per litre for free SO_2 .

A recent investigation of the white wines of France has shown that these limits are a serious hindrance to commerce, and that it is not a fact that it is the sweeter wines which require the most SO_2 for preservation. Wines containing but little sugar frequently contain so much of constituents which combine readily with SO_2 , that if limit quantities are added, the whole is almost at once combined. The leading experts in the Bordeaux white wine trade consider that the 350 mg. per litre allowed for these wines should be raised to 400 mg., with a 10 per cent allowance to meet special cases, without distinction of free or combined SO_2 , or a limit of 100 mg. of free SO_3 per litre without reference to the amount of combined SO_2 .

The total SO_2 may be determined by passing a current of CO_2 through a 400 c.c. flask, the CO_2 entering through one tube through an india-rubber cork, the exit tube leading to a tube containing absorption bulbs. The absorption tube contains 50 c.c. of a 5 per cent solution of iodine (in 7.5 gm. KI per litre). When the air is displaced, 100 c.c. of the sample and 5 c.c. of syrupy phosphoric acid are poured into the flask and the cork at once replaced. The current of CO_2 is allowed to proceed, and after fifteen minutes, the contents of the flask are carefully heated, so that half of the contents distil over

into the absorption tube in the current of CO_2 . The tube should be kept cold by immersion in cold water. The contents of the absorption tube are then poured into a beaker and the sulphuric acid formed by oxidation of the SO_2 precipitated and weighed as barium sulphate. The amount of $\text{BaSO}_4 \times 2.7468$ gives the amount of SO_2 per litre.

The free SO_2 is determined by diluting the wine if red—but not if white—and adding a little sodium carbonate. Excess of dilute sulphuric acid is then added and, with the flask filled with CO_2 , the SO_2 can be titrated with one-twentieth normal iodine, using a little starch water as indicator. The results are approximately accurate. Each c.c. of one-twentieth normal iodine is equivalent to 1.6 mg. of SO_2 .

Colouring Matter.—The examination of the colouring matter of wine is not an easy matter. The following three tests are the French official methods :—

1. Fifty c.c. of the wine rendered alkaline by ammonia are shaken with 15 c.c. of pure amyl alcohol. The amyl alcohol should remain colourless and after separation should remain colourless when acidified with acetic acid.

2. The wine is treated with a 10 per cent solution of acetate of mercury until the precipitate formed does not change colour, when a slight excess of magnesia is added, to render the liquid alkaline. The mixture is then boiled and filtered. The liquid, acidified with dilute sulphuric acid, should remain colourless.

- (3) Fifty c.c. of wine are placed in a porcelain dish and 2 drops of 10 per cent sulphuric acid added, and a small piece of white wool plunged into the liquid. The liquid is boiled for 5 minutes, water being added to replace the loss due to the boiling. The wool is then washed in a current of water. It should then have only the slightest rose-coloured tint, and when dipped into ammonia solution, it should only yield a very pale green colour.

Other useful tests are as follows :—

Five c.c. of a 10 per cent solution of subacetate of lead are added to 20 c.c. of the wine. If the precipitate is of a red-violet colour, it is nearly certain that a vegetable colouring matter has been added—probably that extracted from the berries of *Phytolacca decandra*. If the liquid be heated and then filtered, and amyl alcohol extracts any red colour from the filtrate, the addition of foreign colouring matter is certain.

Test No. 3 alone may be modified by first mordanting the wool by dipping it into a solution of alum and sodium acetate, and the test is then carried out as above. If the colour of the wool is a deep red, aniline colours are present, and this may be confirmed by the behaviour of the wool when treated with ammonia. The pale reddish colour which may result with natural wines is changed to a dirty, pale green. The red due to aniline colours is either unchanged or turned to a yellowish tint, the red colour being restored by washing out the ammonia.

Dupré's gelatine test is a useful one in the hands of one having had experience of the test. Small gelatine cubes are prepared, by soaking 5 grams of gelatine in water and then adding hot water to 100 c.c.

When the jelly has set, it is cut into cubes of about $\frac{3}{4}$ in. on each surface. If one of these cubes be inserted in the sample for forty-eight hours, the colouring matter of pure wine will be found, on cutting sections of the cubes, to have only penetrated just below the surface, most other colouring matters, including aniline reds, cochineal, log-wood, beetroot, litmus, etc., will permeate the jelly and colour it nearly, if not quite, throughout.

There are certain aniline colours which escape the lead acetate test given above. These however are identified by Cazeneuve's oxide of mercury test. Ten c.c. of the sample are shaken with 0.2 gm. of yellow mercuric oxide for at least a minute, and the liquid filtered, preferably after boiling. A clear but coloured filtrate indicates the presence of coal-tar colours—but a colourless filtrate does not prove their absence.

There are other coal-tar colours which may be detected by the following method: Two portions of 100 c.c. each of the wine are extracted with ether, one of them being rendered alkaline by 5 c.c. of ammonia.

The ether from both is evaporated in a porcelain dish with a thread of wool. If the wool in the experiment in which ammonia was used is dyed red, a coal-tar colour was present.

The experiment in which no ammonia was used may give a brownish tint with pure wines, but no pronounced red colour.

The French official test with amyl alcohol should be performed not only on the wine rendered alkaline, but also on the natural and the acidified wine. The following are the inferences to be drawn from the results of these tests.

(1) On the natural wine. A small amount of red colouring matter *may* be extracted from pure wines by amyl alcohol. But it will be changed to a green or blue-green by the addition of ammonia. Any red colour, not so changed, is a powerful indication of added coal-tar colouring matter.

(2) On the alkaline wine. A red extract with amyl alcohol is a fairly certain indication of a coal-tar colour. The ammonia should not be present to a greater extent than 3 c.c. of ordinary strong ammonia (specific gravity = 0.880) per 100 c.c.

(3) On the acidified liquid. Red colouring matter is extracted from pure wines. The amyl alcohol should be shaken with water, and the aqueous solution tested by ammonia, which, in the presence of coal-tar colours, leaves the solution red and not green, or it may be tested by dyeing wool as described above.

The above reactions are sufficient for any ordinary case. Lengthy researches—usually yielding inconclusive results—as to the exact nature of the added colouring matter when present, may be undertaken, but it is rarely necessary to go further than deciding if foreign colouring matter be present or not.

A systematic examination of the colouring matter was published in 1876 by Gautier, and may be consulted in the "Analyst" (xxi. 1).

Tartaric Acid.—The total amount of tartaric acid present (either free or as potassium bitartrate) may be estimated by mixing 20 c.c. of

the wine with 1 c.c. of 10 per cent solution of potassium bromide and 40 c.c. of a mixture of equal volumes of ether and alcohol. The whole is well shaken and left in a closed flask for three days. The liquid is then decanted on to a small filter, which is washed with a little of the alcohol ether mixture. Forty c.c. of warm water is then passed through the filter into the original flask, the contents of which are warmed until the acid tartrate of potassium is dissolved. This is now titrated with one-twentieth normal alkali, using phenol-phthalein as indicator. From the amount of alkali used, the percentage of tartaric acid can be at once calculated, from the following formula, which allows for the solubility correction:—

$$(n \times 0.47) + 0.2$$

gives the number of grms. of tartaric acid per litre, where n = the number of c.c. used.

The genuine character of certain French wines has for many years past been called in question by German chemists on account of their abnormal values for total extract and free tartaric acid. Apart from the fact that many of the wines contain less than the lowest proportion of extract regarded as genuine in Germany, most of them also contain a considerable amount of free tartaric acid. In accordance with German regulations, the free tartaric acid in wines containing not more than 0.8 gm. of total acids in 100 c.c. should not, as a rule, exceed one-fifth to one-sixth of the total non-volatile acids; in wines containing more than 0.8 gm. of total acids per 100 c.c. the proportion of free tartaric may be higher. French chemists, on the other hand, have repeatedly asserted that these wines from the South of France are genuine, and that the free tartaric acid may amount to about 2.0 grms. per litre. In order to obtain complete certainty on the points in dispute, the wines from grapes grown near Barbonne have been examined, and the results show that the frequently high proportion of free tartaric acid must be attributed to the conditions of the soil and not to any additions of that acid. The results on opposite page are typical of those obtained, the amounts of total and free tartaric acid having been estimated by the official German method of Halenske and Möslinger ("Zeit. Anal. Chem." 1905, **34**, 279).

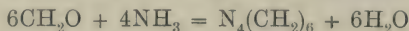
Tannic Acid.—Any one of the modified Löwenthal's processes (see p. 11) may be used for this determination. Ten to twenty c.c. of the sample should be employed, according to the astringency of the wine.

Salicylic Acid.—For the detection of salicylic acid in wine, see pp. 679 *et seq.*

Disinfection of wine barrels is sometimes carried out by means of formaldehyde (paraformaldehyde being volatilized in the barrels for this purpose), and when this is done the formaldehyde is removed by treating the barrels with sodium carbonate solution, water and steam. After this process has been carried out distinct traces of formaldehyde were found in a wine which had been stored in a barrel disinfected with formaldehyde fourteen days previously. For the detection of formaldehyde the reaction described by Arnold and Mentzel ("Analyst,"

Variety of Grapes.	Colour.	Specific Gravity.	Alcohol by Vol. Per cent.	Extract Per cent.	Ash Per cent.	Alkalinity of Ash in c.c. N KOH. I	Total Acids Per cent.	Non-Volatile Acids Per cent.	Sugar Per cent.	Total Tartaric Acid Per cent.	Free Tartaric Acid Per cent.
Aranion											
	1907 White	0.9954	7.7	1.37	0.196	1.55	0.675	0.570	0.100	0.450	0.218
	1907 Red	1.0014	5.2	1.9	0.330	2.75	0.997	0.825	0.120	0.67	0.263
Carignan											
	1907 White	0.9952	9.5	1.90	0.214	2.00	0.675	0.584	0.132	0.270	0.084
	1907 Red	0.9973	9.6	2.34	0.330	2.25	0.825	0.700	0.11	0.375	0.087
Bourret	1907 White	0.9928	11.0	1.55	0.354	2.05	0.487	0.427	0.10	0.150	0.023

xxvii. 227) is recommended, but, as wine frequently contains sulphurous acid, some preliminary treatment is necessary to decompose the formaldehyde-sulphurous acid compound before the test is applied to the distillate of the wine. The wine is distilled after the addition of phosphoric acid, and the distillate is rendered alkaline with potassium hydroxide. After the lapse of fifteen minutes the solution is neutralized with sulphuric acid, and any formaldehyde test is then applied. For the estimation of formaldehyde the method proposed by Legler may be used; it is based on the combination of the formaldehyde with ammonia according to the equation:—



The distillate from a definite volume of the wine is treated with alkali and then neutralized with sulphuric acid, using rosolic acid or litmus as indicator. An excess of standardized ammonia is next added, the mixture is set aside for three hours, and the excess is then titrated with $\frac{\text{N}}{10}$ sulphuric acid. The quantity of formaldehyde present is then

calculated from the ammonia used to combine with the aldehyde.

Succinic Acid.—The best method for the determination of succinic acid in wine is that of Raw ("Zeit. für Anal. Chem." xxxii. 482). One hundred c.c. of the sample are evaporated to a syrupy consistency, repeatedly extracted with boiling alcohol, and the cooled alcoholic solutions filtered, mixed, and distilled. The residue is dissolved in a little hot water, and the cooled solution filtered, if turbid; it is then treated with barium nitrate, 3 to 4 vols. of 90 per cent alcohol are added, and the mixture is well stirred. The precipitate, containing tartaric, malic, and succinic acids, is collected, washed well with 70 per cent alcohol, warmed with sodium carbonate solution, and filtered; the filtrate is neutralized with nitric acid, evaporated to a small bulk, and after neutralization with ammonia, is precipitated with a magnesia mixture, made with magnesium nitrate, ammonium nitrate, and ammonia. The precipitate, which contains the tartaric acid, is filtered off after three or four hours' repose; the filtrate is heated with potash until all the ammonia is expelled, then filtered from magnesia, neutralized exactly with nitric acid, diluted to 100 c.c. to 150 c.c., and precipitated with silver nitrate (1:20). Silver nitrate precipitates succinic acid completely, but produces precipitates in malic acid solutions only when they are stronger than 1:800. The precipitate is collected on a tared filter, washed well dried, and weighed. As a control, it may be ignited and the silver weighed. Should the solution to which silver nitrate is to be added contain chlorides, which may happen if too much alcohol has been added after the barium nitrate, or too long an interval has been allowed before filtration, a portion of it must be evaporated, incinerated, the chlorine determined, and a corresponding quantity of silver chloride subtracted from the weight of the silver succinate.

The method described by Kunz ("Analyst," xxviii. 314) has been subjected to a critical examination, and it is found that while the process as a whole is the best of many methods which have been

proposed for the estimation of succinic acid, certain modifications in the method of procedure are necessary in order to obtain accurate results. The process, as modified by Heide and Steiner, is as follows:—

Fifty c.c. of the wine are evaporated in a basin of about 200 c.c. capacity until the alcohol has been removed; after the addition of 1 c.c. of 10 per cent barium chloride solution and a little phenolphthalein, the residual solution is treated with powdered barium hydroxide until all the acidity has been neutralized. Excess of barium hydroxide is removed by treating the mixture with carbon dioxide, and 85 c.c. of 96 per cent alcohol are then added to the mixture with constant stirring. After the lapse of at least two hours the precipitate, consisting of barium succinate, tartrate, and malate together with other barium salts, is collected on a filter, washed with a small quantity of 80 per cent alcohol, and then washed back again into the basin by the aid of a jet of boiling water. The contents of the basin are now heated until all alcohol has been removed, and 5 per cent potassium permanganate solution is then added in quantities of about 3 c.c. at a time until the red coloration does not disappear after the mixture has stood for five minutes. A further 5 c.c. of potassium permanganate are then added, and the mixture is heated on the water bath for fifteen minutes. The excess of permanganate is destroyed by the addition of sulphurous acid, and after the mixture has been acidified with sulphuric acid, more sulphurous acid is added until all the manganese dioxide has been redissolved. The mixture is then evaporated to a volume of about 30 c.c. and extracted with ether for twelve hours in a percolating apparatus after the addition of so much 40 per cent sulphuric acid that the solution contains about 10 per cent of free sulphuric acid. The ethereal extract is diluted with water, the ether is evaporated, and the residual solution, after neutralization, is transferred to a 100 c.c. flask, 20 c.c. of $\frac{N}{10}$ silver nitrate solution are added and the whole is diluted to the mark and filtered. The excess of silver is then titrated in 50 c.c. of the filtrate, Volhard's method being used for the purpose.

Saccharin may be detected by the method described on page 674.

Identification of Inosite in Natural Wines.—The fact that all natural wines contain inosite affords, according to Perrin ("Ann. de Chem. Anal. Appl." 1909, **14**, 182) a simple means of distinguishing them from artificial products. For the identification of inosite 200 c.c. of the wine are treated with 20 c.c. of basic lead acetate solution and a few drops of alcoholic solution of tannin, and filtered. The filtrate is freed from lead by means of hydrogen sulphide, and the filtrate from the lead sulphide decolorized with animal charcoal and concentrated to about 10 to 20 c.c. on the water bath. The following tests are then applied to this liquid:—

(1) Two drops are heated on platinum foil with one drop of a 10 per cent solution of silver nitrate, and the residue carefully ignited. In the presence of an inosite a violet-rose coloration is obtained. This disappears on cooling, but reappears on again heating the foil.

(2) Two drops of solution are heated on platinum foil with one drop of nitric acid and the carbon incinerated as before. The residue is then treated with a drop of ammonia solution and the liquid again evaporated. A rose coloration which is less pronounced than that obtained in the first test indicates that the wine contained inosite.

Hexamethylene Tetramine in Wines.—The French Minister of Agriculture has recently drawn attention to the fact that hexamethylene tetramine is being used for the purpose of removing sulphites from wine. This is considered to be a fraudulent proceeding and in a circular issued by the Minister the following methods of detecting it are recommended

(1) Strongly acidify a few c.c. of the wine with sulphuric acid, and then add an equal volume of solution of rosanilin bisulphite. An intense violet colour results if this body be present.

(2) Distil 20 c.c. of the wine after acidifying with a few drops of sulphuric acid. Collect the first 5 c.c. add to it 1 c.c. of dilute sulphuric acid and then 5 c.c. of solution of rosanilin bisulphite. An intense violet colour results if hexamethylene tetramine be present.

Numerous other reactions are available for which see Blarez ("Bull. de la Soc. de Pharm. de Bordeaux," 1910, February) and Voisenet ("Ann. de Chim. Anal." 1910, 266).

The Significance of Certain Results in Wine Analysis.—In the author's opinion, too much stress is frequently laid on certain analytical determination on samples of wine. The following are the most important deductions that can be drawn:—

(1) Alcohol. Any excess of alcohol over 14·5 per cent by weight may be regarded as definite evidence of added alcohol. It must be remembered that many wines, especially the sweet wines of Spain and Portugal, are regularly fortified, and must therefore be judged as such, and certain German wines also regularly receive a small addition of alcohol.

(2) Glycerine. Genuine wines usually contain 0·4 per cent to 1 per cent of glycerine but these limits are sometimes exceeded.

(3) Alcohol-glycerine ratio. The researches of Pasteur tended to show that the ratio of alcohol to glycerine in wines undergoing normal fermentation was nearly constant, but later researches have thrown a somewhat different light on the subject. Semichon has shown that where traces of sulphurous acid or fluorides, for example, have been added to the must, less glycerine is developed during the fermentation. Mathieu has shown that the more acid the must naturally is, the greater the amount of glycerine developed. Roos has made exhaustive investigations which appear to prove that the ratio $\frac{\text{alcohol}}{\text{glycerine}}$ shows

great variations, whilst the ratio $\frac{\text{alcohol}}{\text{succinic acid}}$ is far more constant.

It is generally to be found that the alcohol-glycerine ratio of a genuine, unfortified wine, lies between 100 : 5 and 100 : 15 but even these limits are sometimes exceeded.

(4) There is a fair amount of constancy to be observed in the amount of solid extract in wines, so long as they have not been kept

long, if allowance be made for sugar, and, when the wine is plastered, potassium sulphate. Official French standards include a so-called "reduced extract," which is defined as $x - (S - 0.1) - (K - 0.1)$ where x = the percentage of extract, S that of sugar, and K that of potassium sulphate. The "reduced extract" of red wines falls usually between the values 1.8 to 2.6, and of white wines 1.5 to 2.6. Even in old wines the reduced extract rarely falls below 1.5 per cent.

(5) The ratio $\frac{\text{acid}}{\text{alcohol}}$ is used by French chemists to a considerable extent as an indication of watering of wine. Gautier has advocated the sum of the total acids and the alcohol as an indication of watering, as he considers that the free acids are higher as the alcohols are lower, and that the sum of the two is fairly constant; but Hapter considers the ratio $\frac{\text{acid}}{\text{alcohol}}$ to be of greater value. Gautier's value is that usually accepted in France. The figures are expressed by the sum of the alcohol (in per cent by volume) and the acid (as grm. of H_2SO_4 per litre); this figure is not less than 12.5, and for most wines any lower figure is strong evidence of adulteration with water.

(6) The ratio $\frac{\text{alcohol (grm. per 100 c.c.)}}{\text{reduced extract}}$ is regarded with much importance in France.

In genuine, unfortified, red wines this figure never exceeds 4.5 to 4.6, and for white wines 4.8 to 6.5. Any higher ratio indicates the addition of alcohol.

(7) If the ratio indicated in 6 shows that alcohol has been added, then the ratio $\frac{\text{acid}}{\text{alcohol}}$ as shown in (5), must be adjusted to the amount of alcohol naturally present, if the question of added water is to be settled. Thus, if a wine (red) contains 12 per cent alcohol and 1.5 per cent reduced extract, it is obvious that alcohol has been added. If the extract be multiplied by 4.5 the approximate amount of natural alcohol is obtained (by weight). This is then divided by 0.8 to give the percentages by volume. To this value—8.5—the acidity in grm. of H_2SO_4 per litre is added, and if the total be less than 12.5 it may be inferred that water has also been added.

MALT LIQUORS.

Beer, including ale, stout, etc., in this country is not restricted, as it is in Bavaria, for example, to the product of fermentation of a mixture of barley malt, hops, and water, by the aid of yeast. It is more properly described as the product of the fermentation of a saccharine infusion, suitably bittered by a harmless bitter substance.

Many of the best brands of beer, however, are brewed from nothing but malted barley and hops, so that some account of malt is necessary.

In the preparation of malt, the grain—barley or other grain—is well steeped in water and after fermentation, it is dried and heated, or cured, in a kiln. The principal changes taking place during this pro-

cess are an increase in the amount of soluble carbohydrates at the expense of the starch, and a conversion of the insoluble nitrogenous matter into a more soluble form. The following figures illustrate the composition of barley malt. They are due to O'Sullivan and are both pale malts :—

	1.	2.
	Per cent	Per cent
Starch	44.15	45.13
Other carbohydrates (of which 60 to 70 per cent is fermentable sugar)	21.23	19.39
Cellular tissue	11.57	10.09
Fat	1.65	1.96
Albumenoids—		
(a) Soluble in alcohol of sp. gr. 0.820, and in cold water	0.63	0.46
(b) Soluble in cold water and at 68°	3.23	3.12
(c) Insoluble in cold water but soluble at 68° to 70°	2.37	1.36
(d) Insoluble at 68° to 70° but soluble in cold water	0.48	0.37
(e) Insoluble in cold water and at 70°	6.38	8.49
Ash	2.60	1.92
Water	5.83	7.47

Well-malted barley is of a yellowish colour, unless it has been heated in order to partially caramelize the sugar, in order to obtain a high-coloured malt such as is used for stouts and porters.

Good malt should float on cold water. It should not be too hard, being easily crushed between the fingers, but at the same time should be crisp. The acrospire should be from two-thirds to three-fourths of the length of the grain, but should in no case protrude, as too much albuminoid matter would be extracted in the washing. Malt dried at about 32° to 36° is pale in colour and is used for the palest grades of beer. Malt dried at from 38° to 50° is used for the various grades of beer up to very dark brown beers, whilst much higher temperatures are employed when the malt is used for black beers.

The principal object in malting is to produce a relatively large amount of diastase, a ferment capable of converting starch into the soluble carbohydrates, maltose, and dextrin. Malt, however, contains far more diastase than is necessary to convert the starch contained therein into maltose, so that for many beers, considerable amounts of other grain, such as rice, are added to a small quantity of malt.

The Valuation of Malt.—The quantity and nature of the water-soluble constituents of malt, together with its diastatic value, are the principal chemical criteria of its quality.

The following may be regarded as standard methods for this country (being recommended by a special committee appointed by the Council of the Institute of Brewing (*vide* "Jour. Inst. of Brewing," 1906, 12).

In the report of this committee it is recommended that samples should be from 10 per cent of the number of sacks, and the samples

should be taken from a depth of at least six inches below the surface. The samples should then be ground in a Seck mill set at 25°. Only enough for each determination is ground at a time, and the determination at once proceeded with.

Extractive Matter.—Three hundred and sixty c.c. of distilled water heated to 155° F. are mixed with 50 grms. of the ground malt, in a beaker of about 500 c.c. capacity. The beaker is kept covered with a clock glass and kept at 150° F. for fifty-five minutes in a warm water bath. The temperature is then raised to 158° in five minutes, and the mixture washed into a flask graduated to 515 c.c. (the volume of the insoluble part if 50 grms. of malt are assumed to occupy a volume of 15 c.c.), the whole cooled to 60° F., made up to the mark with water, well shaken and filtered through well-ribbed filter paper.

The amount of extract in the clear wort is now deduced by taking the specific gravity, deducting 1000 (water=1000) and dividing the remainder figure by 4. This gives the grms. of extract in 100 c.c. of the wort. [The figure 4 is not universally accepted, figures varying from 3·8 to 3·95 being used by some workers.]

In the brewing trade, the value of worts is usually expressed in pounds per barrel, this being the number of lb. in excess of 360 contained in a barrel of 36 gallons. An instrument used largely in this connexion is the hydrometer known as Bates' saccharometer, which is graduated to read lb. per barrel directly. These readings are convertible into specific gravities by dividing the value by 0·36 (or multiplying by 2·778) and then adding 1000. Thus a barrel of wort weighing 380 lb., is said to have a saccharometric value of 20 lb. per barrel. The specific gravity of this would be 1055·5 (water=1000) since $\frac{360}{380} = \frac{1000}{1055\cdot5}$; and from the above-given figures it would contain 13·8 grms. of residue per 100 c.c. or 50·1 lb. per barrel. And a wort of specific gravity 1·055 (the standard strength at which the duty per barrel is levied) has a saccharometric value 19·80 (1055 - 1000) × 0·36. The above considerations, of course, apply to unfermented worts.

The solid matters of worts consist largely of maltose, with dextrans, albuminoids, ash, soluble starch, etc.

The Colour of the Wort.—The colour of the wort is a matter of importance to the brewer's chemist, but has no particular interest in connexion with the analysis of the finished product. For particulars of this reference should be made to the "Journal of the Institute of Brewing" (1906, 12, 302 and 1907, 13, 26).

Moisture.—About 5 grms. are dried at 99° to 100° for five hours in a shallow dish.

Diastatic Value.—Ling's method of carrying out the determination of the diastatic value (Lintner value) yields most concordant results. Twenty-five grms. of ground malt are exhausted with 500 c.c. of absolutely pure distilled water at 70° F., and filtered. When the filtrate is perfectly clear, 3 c.c. are allowed to react with 100 c.c. of a 2 per cent solution of soluble starch at 70° F. for one hour, in a 200

c.c. flask. The starch solution is prepared by digesting pure potato starch with HCl (sp. gr. 1.037) for a week at ordinary temperatures, stirring well each day. Two grms. of acid should be used for each gm. of starch. The powder should then be repeatedly washed with water until free from acid, freed from water as far as possible by means of an exhaust filter, and allowed to dry on a clean unglazed tile. It is then dried at about 110° F. for a short time. Two grms. of this are then dissolved in 100 c.c. of boiling water.

At the end of the hour's reaction of the malt extract and soluble starch, 1 c.c. of normal potash solution is added to stop further action, the liquid cooled to 60° F. and made up to 200 c.c. It is then titrated in the following manner: Five c.c. of Fehling's solution are measured into a 150 c.c. flask and heated to boiling. The solution of starch (converted) is then run in 5 c.c. at a time, the liquid being well shaken and kept at boiling temperature. After each addition, the liquid is well boiled, a drop is withdrawn by a glass rod, and brought into contact with a drop of ferrous thiocyanate, spotted on a white tile. So long as any cupric salt remains the red colour of ferric thiocyanate is at once developed, so that the end reaction is well marked. The results are to be calculated by the formula $A = \frac{1000}{xy}$, where A = the diastatic activity in the empirical Lintner de-

grees, x = the number of c.c. of malt extract prepared as above described in 100 c.c. of the fully diluted (i.e. to 200 c.c.) converted starch liquid, and y = the number of c.c. of the solution necessary to reduce 5 c.c. of Fehling's solution.

For malts showing a higher value than 50, 2 c.c. only of the malt extract—or if the value be over 80, only 1 c.c.—should be used. An alternative method of determining the Lintner value is described under Extract of Malt, which, as a matter of convenience, is described with the carbohydrate food stuffs.

Cold Water Extract.—There is considerable difference of opinion as to the value of this figure, some chemists holding that if a malt has been forced in its rate of sprouting, too much starch is converted into soluble sugar; other chemists see no objection to the use of a forced malt. Twenty-five grms. of ground malt are digested with 250 c.c. of distilled water containing 2 c.c. of normal solution of ammonia, for three hours at 70° F., with occasional stirring. The specific gravity of the filtrate is then taken and the excess of this figure over 1000, multiplied by 10 and divided by 3.86, gives the percentage of extract, which averages 16 to 20 per cent. The correction in specific gravity due to the trace of ammonia is practically negligible.

Nitrogenous Matter.—If this value is required, it should be determined in the usual manner by Kjeldahl's method (p. 403). The average, based on the multiplying factor 6.25, is 10 to 11.5 per cent.

Arsenic.—The determination of arsenic may be carried out as described under drugs (pp. 662 *et seq.*).

The wort, either from the malted barley or a mixture of this with other grain, is boiled in order to concentrate and utilize it, when hops

or other bitter material are added to it, and the boiling continued. After cooling the clear liquid is run into fermenting vats where properly selected yeast, usually *Saccharomyces cerevisiae*, but sometimes other species, is added, and alcoholic fermentation is allowed to proceed. If the fermentation be conducted at low temperatures, from 4° to 10° C., it is a slow process and yeast settles at the bottom of the liquid. If the process be conducted at 15° to 22°, quick fermentation goes on and most of the yeast rises to the surface and is skimmed off. Considerable differences in the finished beer result according to the condition of fermentation. The following are the appearances of the top and bottom yeasts which grow in the two processes of fermentation.

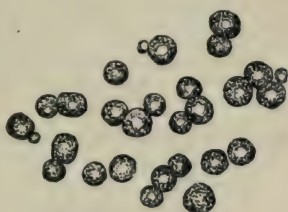


FIG. 37.—Top yeast.



FIG. 38.—Bottom yeast.

In England the following are the principal varieties of beer brewed :—

Beer is generally used to indicate the pale to amber-coloured ale, as distinguished from stout.

Stout is the black variety of beer, prepared by the use of a caramelized malt.

Porter is a weak variety of stout.

There are several varieties of "lager" beer brewed in this country, in imitation of German beers. But in the author's opinion (based on experience as a juror in the beer section of the Paris Exposition, 1900), no English-brewed "lager" beer is identical in character with the high-grade German lager beers. The distinction in the varieties of German beer are not to-day of so much importance as they were formerly, owing to the improvements in methods of storage, refrigeration, etc.

"Schenck" or winter beer is a quickly fermented beer made for immediate use, containing a low proportion of alcohol, so that it will become acid by keeping.

"Lager" or "summer" beer, so named because it is stored—*lager* = a store-house—is higher in alcohol than schenck beer, and will keep for a much longer time; formerly it was brewed in the winter and kept for summer use.

Bock beer occupied an intermediate position and was a fairly strong beer which would keep longer than schenck beer.

Export beer is the strongest variety of lager beer made, but is

usually pasteurized before it is sent abroad, where it is consumed fairly rapidly.

The following represent the compositions of a number of typical beers examined by König :—

	Sp. Gravity.	Water.	CO ₂	Alcohol by Weight.	Extract.	Albumenoids.	Sugar.	Dextrin.	Glycerin.	Acetic Acid.	Ash.	F ₂ O ₅ .
Schenk beer	1.0144	91.11	0.197	3.36	5.34	0.74	0.95	3.11	0.120	0.156	0.204	0.055
Lager "	1.0162	90.08	0.196	3.93	5.79	0.71	0.88	3.73	0.165	0.151	0.228	0.077
Export "	1.0176	89.01	0.209	4.40	6.38	0.74	1.20	2.47	0.154	0.161	0.247	0.074
Bock "	1.0213	87.87	0.234	4.69	7.21	0.73	1.81	3.97	0.176	0.165	0.263	0.089
Ale . "	1.0140	88.00	0.200	5.00	6.40	0.54	0.95	1.70	0.250	0.260	0.300	0.160
Porter .	1.0200	88.10	0.190	4.90	9.60	0.60	2.40	2.80	0.240	0.250	0.340	0.085

Some strong Burton and Scotch ales will contain as much as 12 per cent to 14 per cent of extract and from 8 per cent to 10 per cent of alcohol. Porter rarely contains more than 6 per cent to 7 per cent of alcohol, the sample examined by König being exceptionally low.

The ash of beer has the following average composition :—

	Per cent
Soda	8.94
Potash	33.67
Lime	2.78
Magnesia	6.24
Fe ₂ O ₃	0.48
P ₂ O ₅	31.35
Chlorine	2.93
SO ₃	3.47
SiO ₂	9.29

The physical differences as indicated by taste are of more importance than the chemical characters in discriminating the various qualities of beers. The typical differences between English beers and German beers are due to the fact that English beers are generally prepared by a top fermentation at a more elevated temperature than that employed in the fermentation of German beers, which are produced by bottom fermentation. The yeasts used, too, are different varieties. The German beers contain less alcohol, but more dextrin, sugar and nitrogenous substances than English beers.

The Analysis of Beer.—The duty on beer is calculated from the strength of the unfermented wort as indicated by its specific gravity. So that where beer is exported and a rebate of duty claimed, it becomes a matter of importance for the analyst to be able to determine from the finished beer what this value was. Since the amount of alcohol is about 50 per cent of the sugars fermented, it is clear that a determination of the alcohol will give the means of determining the original specific gravity of the wort. The reduction of the specific

gravity by fermentation is known technically as the "attenuation" of the wort.

To obtain this figure the specific gravity of the beer freed from alcohol by evaporation and made up to its original volume, and the specific gravity of the alcohol distilled from the beer, made up to its original volume, are taken.

The beer is first freed as much as possible from CO_2 by pouring from one vessel to another and filtering through either cotton wool or paper and then 100 c.c. are diluted with 40 c.c. of water, and about 80 c.c. distilled. Both the distillate and the residue are made up to 100 c.c. and the specific gravities taken.

The distillate now represents the fermented matter as a mixture of alcohol and water, whilst the residue indicates the unfermented matter left from the original wort. The specific gravity of the distillate is subtracted from 1000 (specific gravity of water) and the difference is called the degree of spirit indication.

From the table compiled by Graham, Redwood and Hobhouse which is legalised for use by the excise in this country, the degree of specific gravity lost by the fermentation is found, and this figure when added to the "extract gravity"—i.e. the gravity of the de-alcoholized beer made up to its original volume—gives the specific gravity of the original wort. The table is as follows:—

Degree of Spirit Indication.	Fractions of a Degree of Same.									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	—	0.3	0.6	0.9	1.2	1.5	1.8	2.1	2.4	2.7
1	3.0	3.3	3.7	4.1	4.4	4.8	5.1	5.5	5.9	6.2
2	6.6	7.0	7.4	7.8	8.2	8.6	9.0	9.4	9.8	10.2
3	10.7	11.1	11.5	12.0	12.4	12.9	13.3	13.8	14.2	14.7
4	15.1	15.5	16.0	16.4	16.8	17.3	17.7	18.2	18.6	19.1
5	19.5	19.9	20.4	20.9	21.3	21.8	22.2	22.7	23.1	23.6
6	24.1	24.6	25.0	25.5	26.0	26.4	26.9	27.4	27.8	28.3
7	28.8	29.2	29.7	30.2	30.7	31.2	31.7	32.2	32.7	33.2
8	33.7	34.3	34.8	35.4	35.9	36.5	37.0	37.5	38.0	38.6
9	39.1	39.7	40.2	40.7	41.2	41.7	42.2	42.7	43.2	43.7
10	44.2	44.7	45.1	45.6	46.0	46.5	47.0	47.5	48.0	48.5
11	49.0	49.6	50.1	50.6	51.2	51.7	52.2	52.7	53.3	53.8
12	54.3	54.9	55.4	55.9	56.4	56.9	57.4	57.9	58.4	58.9
13	59.4	60.0	60.5	61.1	61.6	62.2	62.7	63.3	63.8	64.3
14	64.8	65.4	65.9	66.5	67.1	67.6	68.2	68.7	69.3	69.9
15	70.5	71.1	71.7	72.3	72.9	73.5	74.1	74.7	75.3	75.9

For example, if the "extract gravity" be 1.0420, and the specific gravity of the alcohol distillate 0.9905; then the degree of spirit indication is $1000 - 9905 = 9.5$. From the table the corresponding degree of gravity lost is 41.7. So that $1.0420 + 0.0417 = 1.0837$ is the original gravity of the wort.

These values are based on the presence of about 0.1 per cent of free acid calculated as acetic acid in the beer. In the case of sour

beers or beers containing much free acid, a correction must be added to the apparent spirit indication. This may be obtained from the following table, which allows for the conversion of the small quantities of alcohol into acetic acid.

Excess of Acetic Acid over 0.1 Per cent.	$\frac{1}{100}$ of a Per cent.									
	·00	·01	·02	·03	·04	·05	·06	·07	·08	·09
0	—	·02	·04	·06	·07	·08	·09	·11	·12	·13
·1	·14	·15	·17	·18	·19	·21	·22	·23	·24	·26
·2	·27	·28	·29	·31	·32	·33	·34	·35	·37	·38
·3	·39	·40	·42	·43	·44	·46	·47	·48	·49	·51
·4	·52	·53	·55	·56	·57	·59	·60	·61	·62	·64
·5	·65	·66	·67	·69	·70	·71	·72	·73	·75	·76
·6	·77	·78	·80	·81	·82	·84	·85	·86	·87	·89
·7	·90	·91	·93	·94	·95	·97	·98	·99	1·00	1·02
·8	1·03	1·04	1·05	1·07	1·08	1·09	1·10	1·11	1·13	1·14
·9	1·15	1·16	1·18	1·19	1·21	1·22	1·23	1·25	1·26	1·28
1·0	1·29	1·31	1·33	1·35	1·36	1·37	1·38	1·40	1·41	1·42

The acetic acid for the purposes of this correction should be determined by deducting the amount of free *fixed* acids as determined by titration of the dried residue of the beer redissolved in water from the total free acids, both being calculated as acetic acid. Decinormal solution of ammonia, using red litmus as indicator, should be used for the titrations.

For example, if in the above illustration the free acidity, of the beer (volatile acidity) equal 0.33 per cent (i.e. 0.23 in excess of 0.1 per cent) then the above table gives the correction as .31. This is added to the apparent spirit indicator value, namely 9.5, which is now 9.81. From the former table this figure gives a "specific gravity lost" of 43.2, which, added to the extract gives 1.0852 as the original gravity of the wort.

An alternative method for determining the original specific gravity of the wort consists in determining the "spirit indication" by merely deducting the specific gravity of the beer itself from that of the beer from which the alcohol is expelled, made up to original bulk with water. The alcohol need not be collected if this determination is made.

The calculations and corrections for acidity are identical with the former method, except that when the "gravity lost" is found from the table one-fortieth of the figure found must be added as a correction. For example:—

Specific gravity of the de-alcoholized beer made up to volume.	1.046
Specific gravity of the beer	1.035
Difference = spirit indication	11
Specific gravity lost (from table)	49
Add one-fortieth	1.22
Corrected "gravity lost"	50.22
Add "extract gravity"	1.046
Original gravity of wort	1.0692

The following determinations on the finished beer are at times necessary. Alcohol, extractive, residual fermentable matter, acids, glycerine, reducing sugars and dextrin, proteids, phosphoric acid, chlorine, carbonic acid, detection of bitter principles, arsenic, and preservatives.

Alcohol and Extract.—The alcohol may be determined by distilling 80 per cent of the beer freed as much as possible from CO_2 and making the distillate up to original volume, whence the alcohol is deduced from the specific gravity by the table on p. 275. The extract is not accurately determined by evaporation as maltose is dehydrated at temperatures over 75°C . Unless very accurate results are required, however, drying at 100° gives fairly approximate results. A fairly accurate method is to de-alcoholize the beer, make up to original volume, and take the specific gravity of the liquid. The excess of 1000 of this value, divided by 4, gives the amount in grms. of dry residue per 100 c.c. It is probable that the divisor should be a little lower than this, but 4 is usually accepted, and gives fairly accurate results.

An accurate method for the determination of the alcohol and the extract in beer depends on the determination of the refractive index of the beer itself (R) and of the distillate from the beer, made up to original volume (R'). For full details of this the following papers may be consulted: Ackermann and Steinmann ("Zeit. Gesamt. Brau.," 1905, **28**, 259 and 1906, **29**, 146); Ling and Pope ("J. Fed. Inst. of Brewing," 1901, **7**, 170) and Race ("J. Soc. Chem. Ind." 1902, **27**, 544).

Residual Fermentable Matter.—One hundred c.c. of the beer are heated to drive off the alcohol, made up to original volume with water and fermented with 1 to 2 grms. of pressed yeast for forty-eight hours. The re-fermented liquid is boiled to drive off alcohol, made up to original volume with water and a little cream of alumina and filtered. The "maltose" is now determined by reduction of Fehling's solution (p. 156) in both this liquid and in the original beer itself, first deprived of its alcohol. The difference between the two values gives the amount of residual fermentable matter in the original beer, in terms of maltose (but in reality including degradation products of maltose).

Free Acids.—The beer is freed from carbonic acid by pouring from vessel to vessel and filtering through paper or wool, and then titrated with $\frac{N}{10}$ solution of soda or ammonia using litmus as indicator. The total acidity is usually expressed as lactic acid, each c.c. of $\frac{N}{10}$ alkali being equal 0.009 gm. of lactic acid. The fixed acidity is determined by evaporating to dryness and titrating the residue after re-solution in water. This is expressed as lactic acid. The difference between the two values is calculated to acetic acid (1 c.c. $\frac{N}{10}$ alkali = 0.006 gm. acetic acid) and expressed as such.

Glycerin.—This is determined, when necessary, as directed under wine (p. 326), except that the milk of lime is added after the CO_2 is

expelled; and it is advisable to use a further portion of alcohol and ether in the extraction.

Reducing Sugars and Dextrin.—Fifty c.c. of the beer are diluted to 200 c.c., heated on a water bath for two and a half hours with 20 c.c. of HCl (specific gravity 1.13), almost neutralized by soda solution, made up to 300 c.c. when cold, filtered and the dextrose determined by reduction of Fehling's solution.

Determine the amount of reducing sugars in the beer by a direct reduction of Fehling's solution, and calculate as maltose. Multiply this value by 0.95 (to convert to dextrose equivalent) and subtract the product from the amount of dextrose after the above inversion, calling the result the dextrose derived from dextrin. Multiply this figure by 0.9 which gives the dextrin in the beer.

Proteids.—Fifty c.c. are treated with 5 c.c. of dilute H_2SO_4 , and then concentrated to a syrup. The nitrogen is then determined by Kjeldahl's process, and multiplied by 6.25 to give the proteid value.

Phosphoric acid and other mineral constituents. The principal value in the determination of the mineral constituents of a beer lies in the fact that owing to the characters of a given water supply it is often possible to decide whether a given beer is the product of a given brewery. Further by English excise regulations no excess over 50 grains of sodium chloride per gallon is allowed in beer. The phosphoric acid may, except in very dark beers, be determined by titration with uranium acetate solution in the usual manner. The uranium solution should be standardized so that 20 c.c. corresponds to 0.1 grm. P_2O_5 , using potassium ferrocyanide in spots on a white tile as indicator. Fifty c.c. of the beer are titrated, each c.c. of uranium solution being equivalent to 0.01 per cent of P_2O_5 .

In dark beers, the ash must be moistened with HCl, dried and boiled with 50 c.c. of water, which is then titrated as above.

All malt beers contain the highest amount of P_2O_5 .

The chlorides are determined by evaporating 50 c.c. of beer, with a little Na_2CO_3 , and incinerating at as low a temperature as possible, until a black ash is obtained, which is extracted with water in the usual manner, and the chlorine titrated with $\frac{\text{N}}{10}$ silver nitrate.

The determination of sulphates is often of importance from the point of view indicated above. It is important, in making this determination, that the beer should be evaporated in the presence of a little sodium hydroxide, or there will be a loss of SO_2 .

Carbonic Acid.—The determination of CO_2 is not often required, but if it is, it is obvious that the case of beers bottled with the common screw stopper cannot be dealt with, as the act of opening the bottle causes an immediate loss of the gas. In other cases a metal champagne tap may be inserted through the cork, and this is connected by rubber tubing with (1) an empty safety flask whose exit tube passes on to a series of four absorption tubes, of which the first three contain (1) calcium chloride (2) sulphuric acid, and (3) sulphuric acid, in order to absorb moisture, and (4) concentrated solution of caustic potash in which the CO_2 is absorbed. The tap is turned on

slightly so that the gas bubbles through the first three absorption tubes slowly, and when no more gas is given off the water bath in which the bottle is standing is heated gradually, so that all the CO_2 is driven off. The difference between the weights of the CO_2 bulb after and before the process gives the amount of CO_2 .

The Bitter Substances in Beer.—Most of the bitter substances alleged to have been found in beer are, to-day at all events, apocryphal. Alkaloids—apart from cases of poisoning—are rarely if ever to be found in beer. The absence of a precipitate with any of the usual alkaloidal reagents (see p. 502) is sufficient guarantee of the absence of added alkaloids.

Quassia, chiretta, gentian, and aloes may be present, the former two especially. Since the bitter principle of hops is readily soluble in ether, it follows that if a sample of beer be evaporated to a syrupy consistence and extracted with ether, and the ether separated and evaporated, the absence of a bitter taste in the ether extract is proof of the *absence* of hops; whilst the presence of a bitter taste is *not* proof of the presence of hops.

The following methods will usually be found sufficient, in addition to the above, for the examination of the bitter principles in beer. The bitter principle of hops is completely precipitated by solution of lead acetate or subacetate, so that the filtrate after such treatment, when concentrated, has no bitter taste unless some other bitter be present. The filtrate should be treated with H_2S to remove excess of lead, and then concentrated to a syrup. If ether extracts anything with a bitter taste from this concentrated filtrate, it is certain that a foreign bitter has been used.

Chapman ("Analyst," xxv. 35) proposes to differentiate between hops and quassia by the following process: 500 c.c. of the beer are evaporated to dryness with a little sand, the whole being constantly stirred, and the residue is dried in an air oven, and powdered. It is then extracted with ether and after the ether is evaporated, the residual extract is oxidized by the careful addition of an alkaline solution of potassium permanganate (40 grms. of permanganate and 10 grms. of KOH per litre). This should be added gradually with shaking and warming. When the permanganate is but slowly reduced, a few drops of hot solution of oxalic acid are added, which decolorizes the slight excess of permanganate, and the colourless liquid is filtered and evaporated to dryness. The dry residue is then treated with dilute sulphuric acid, when, if hops be present, the distinctive odour of valerianic acid is evolved. Neither chiretta nor quassia give this result, whereas chamomiles give a distinct valerianic acid reaction.

If quassia be present, the chloroform extract from the beer (rendered slightly acid with H_2SO_4) when dried, gives, with a weak alcoholic solution of ferric chloride, a distinct mahogany brown colour; or, if the residue be treated with bromine and ammonia, a bright yellow colour is given.

Chiretta is indicated by the ether residue giving a straw colour, changing to a dull purple-brown, with bromine and ammonia. The chloroform residue does not yield this reaction.

Gentian is indicated by the chloroform residue (from acidified beer) yielding a carmine-red colour when treated with warm concentrated H_2SO_4 . A trace of ferric chloride converts this into a green-brown colour.

The bitter principle of aloes is indicated by treating the dried residue of 200 c.c. of beer with warm ammonia, and filtering and cooling the resulting liquid and then adding HCl. Aloe resin is precipitated and is collected. It is insoluble in ether, chloroform or petroleum ether, but soluble in alcohol. Its taste and odour render it easy for identification when so collected.

The following outline of processes for the examination of the bitter substance in beer is due to Allen (Vol. I, 4th edition, p. 162, Baker).

One thousand c.c. of beer are evaporated to 500 c.c. and neutral lead acetate solution added : the liquid is boiled for fifteen minutes and filtered hot. If any precipitate separates on cooling, the liquid is again filtered.

<p><i>Precipitate</i> contains hop bitter and chiretta bitter.</p>		<p><i>Filtrate.</i> Remove Pb by H_2S; filter : concentrate to 150 c.c. and taste. If any bitter taste remains, the liquid is acidified with dilute H_2SO_4 and repeatedly shaken with $CHCl_3$.</p>	
<p><i>Chloroform layer</i>, on evaporation, leaves a bitter extract in the case of gentian, calumba, quassia (and <i>old</i> hops) and only slight bitter taste with chiretta. Residue is dissolved in a little alcohol, hot water added and the hot solution treated with NH_3 and basic lead acetate and filtered.</p>		<p>Aqueous liquid is now well shaken with ether.</p>	
<p><i>Precipitate</i> contains the bitters of <i>old</i> hops, gentian or caramel. It is suspended in water, decomposed by H_2S, and the solution shaken with $CHCl_3$.</p>		<p>Filtrate boiled to remove NH_3, slight excess of H_2SO_4 added, the liquid filtered and tasted. If bitter it is shaken with $CHCl_3$ and the residue tasted. If bitter it indicates calumba or quassia.</p>	
<p><i>Chloroform layer</i> contains gentian, or <i>old</i> hop bitter.</p>		<p><i>Aqueous liquid</i> is, if still bitter, rendered alkaline and extracted with ether-chloroform. A bitter extract indicates calumba or strychnine.</p>	
<p><i>Aqueous layer</i> contains traces of caramel bitter.</p>		<p>Ether layer leaves bitter residue in the case of chiretta, gentian or calumba. It is dissolved in a little alcohol, hot water added, and the NH_3 and basic lead acetate solution. It is then filtered.</p>	
		<p>Precipitate suspended in water and decomposed by H_2S. If filtrate is bitter, gentian is indicated.</p>	
		<p>Filtrate is treated with slight excess of H_2SO_4 and tasted. Bitter taste indicates calumba or chiretta.</p>	

Saccharin.—This may be searched for as described under saccharin (p. 674).

Preservatives.—Salicylic acid may be searched for as described under that acid (p. 679).

Sulphites are sometimes present in beer, and may be searched for by adding 5 c.c. of phosphoric acid to 500 c.c. of beer, and distilling 250 c.c. of the liquid. The SO_2 present is estimated by titrating with one-hundredth normal iodine solution. One c.c. = 0.00032 grm. SO_2 . The presence of SO_2 may be confirmed by precipitating the oxidized SO_2 in this distillate by $BaCl_2$ in the presence of HCl.

Fluorides are occasionally to be found in beer. To detect this 200 c.c. of the sample are rendered alkaline with ammonium carbonate, boiled with 2 c.c. of a 10 per cent calcium chloride solution, for ten minutes, and the precipitate collected, washed, and dried. The dried precipitate is ignited (without the paper) in a platinum crucible, powdered, and moistened with 2 or 3 drops of water and 1 c.c. of strong H_2SO_4 . The crucible is covered with a watch-glass which is coated with wax and the wax cut through to the glass with a style. The crucible is now warmed on a water bath, and if fluorides are present the glass will be etched. The wax is kept from melting by coating the *convex* surface, and placing pieces of ice on the upper concave surface.

Boric acid may be found as described under milk (p. 60).

CIDER.

Cider is understood to be the product of alcoholic fermentation of apple juice.

Much cider is made from bruised fruit, and, especially in America, with but little care as to the cleanliness employed during the fermentation process. In such cases it is necessary to use a preservative, or the cider will rapidly turn to vinegar. France has paid considerable attention to the manufacture of cider, and most experts consider that French ciders are the best obtainable.

There is no doubt that the absence of a legal definition of cider in this country has materially prejudiced the development of the industry in a healthy manner. In France the legal position is as follows (Durham, "Journal of the Royal Institute of Public Health, May 1908") : "No drink may be sold as cider unless it is the product of fermentation of the juice of fresh apples, unless it contains a certain proportion of certain chemical constituents, and unless not less than a given proportion of apples has been used to produce a given volume of fluid. Moreover it must not contain any artificial sweetening chemicals such as saccharin; it must not contain any chemical flavouring additions; the employment of certain antiseptics (as sulphurous acid) may be permissible within defined limits, or entirely prohibited (as borates). Artificial colouring agents are likewise prohibited.

Much of the modern systematic work on the analyses of cider is due to A. H. Allen ("Analyst," xxvii. 183). The following represent the compositions of several varieties of apples examined by Allen :—

	Water.	Free Acid (as malic).	Glucose.	Sucrose.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent
Table apples	81.62	0.88	9.28	6.28	0.44
Cooking apples	84.74	0.56	8.75	2.29	0.33
Cider apples	80.29	0.097	9.43	2.95	0.54
" "	84.14	0.36	7.21	2.84	0.44

The following represent the composition of apple (and incidentally of pear) juice, as recorded by Truelle :—

	Apple Juice.		Pear Juice.	
	Per cent		Per cent	
Sp. gravity	1.057 to	1.1110	1.067 to	1.098
Total solids	14.94	28.57	17.53	25.32
Acidity (as H_2SO_4)	0.07	0.74	0.08	0.24
Sucrose	0.56	7.17	1.67	6.14
Glucose	10.84	18.18	10.81	20.99
Tannin	0.026	0.81	0.10	0.32

Allen gives the following figures for apple juice prepared in England. The analyses were made about thirty-six hours after the apples were pressed, so that a little alcohol was found :—

	Per cent	Per cent	Per cent	Per cent
Sp. gravity	1.0550	1.0530	1.0470	1.0470
Alcohol by weight	0.10	—	1.04	1.13
Solids	14.63	12.74	11.91	11.95
Glucose	13.51	10.48	9.13	8.82
Sucrose	1.34	0.69	0.66	0.38
Fixed acid, as malic	0.28	0.42	0.45	0.50
Ash	0.35	0.30	0.22	0.26
Tannin	—	0.22	—	—

The following tables show the average composition of the apple and its juice, cider, and cider vinegar, as recorded by C. A. Brown (" Jour. Amer. Chem. Soc." 1901, xxiii. 809) :—

	Water.	Reducing Sugars.	Sucrose.	Starch.	Ash.	Fixed Acid (as Malic).
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Unripe apples	80.67	6.43	2.84	3.92	0.27	1.14
Summer apples	85.00	7.10	3.36	1.04	0.28	0.68
Winter apples	82.16	8.16	4.16	—	0.26	0.59

	Sp. gr.	Solids.	Reducing Sugars.	Sucrose.	Acid as Malic.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Summer apple juice	1.0502	12.29	6.76	3.23	0.72	0.12
Winter apple juice	1.0569	13.96	8.57	3.40	0.43	0.12
Cider	1.006	2.34	0.32	—	0.25	0.04
Cider vinegar	1.0184	2.00	0.52	—	0.14	0.01

Brown also gives the following analyses of five typical samples of cider :—

	Sp. gr.	Solids.	Reducing Sugars.	Malic Acid.	Acetic Acid.	Alcohol.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	0.9981	1.94	0.19	0.21	0.24	6.85	0.25
2	1.0012	2.71	0.19	0.24	0.42	5.13	0.32
3	1.0052	3.26	0.89	0.30	0.48	4.67	0.29
4	1.0007	1.93	0.34	0.27	0.21	4.95	0.23
5	1.0051	2.71	0.24	0.29	1.96	4.26	0.36

Allen publishes (*loc. cit.*) numerous analyses of English ciders of which the following are the average figures :—

Grams. per 100 c.c.			
	Norfolk Bottled.	Devonshire Bottled.	Draught Ciders.
	Per cent	Per cent	Per cent
Sp. gravity	1.002 to 1.012	1.003 to 1.032	1.006 to 1.028
Alcohol by weight	5.3 „ 7.69	2.57 „ 5.39	2.49 „ 5.86
Extractive	2.07 „ 5.47	2.12 „ 7.93	2.59 „ 7.63
Glucose	0.77 „ 4.55	0.94 „ 7.24	0 „ 4.17
Fixed acid as malic	0.31 „ 0.42	0.12 „ 0.35	—
Volatile acid as acetic	0.07 „ 0.21	0.19 „ 0.37	0.2 „ 0.43
Ash	0.26 „ 0.33	0.23 „ 0.36	0.16 „ 0.23

There is no evidence as to whether these ciders were genuine or not. Roques ("Le Cidre," p. 128) gives the following as the results of numerous analyses of French ciders :—

	Maximum.	Minimum.	Mean.
	Per cent	Per cent	Per cent
Sp. gravity	1.041	1.0012	1.0159
Alcohol per cent	6.5	3.5	5.2
Sugar free extract per cent	6.46	2.262	3.39
Sugar per cent	6.08	traces	2.162
Ash per cent	0.432	0.248	0.326
Alkalinity of ash (K ₂ CO ₃) per cent	0.368	0.204	0.256

Kulisch ("Land. Jahrb." 19, 83) gives the following as the figures for German ciders :—

		Minimum.	Maximum.
		Per cent	Per cent
Specific gravity	. .	0.9977	1.050
Alcohol per cent	. .	5.4	7.3
Total extract	. . .	1.923	3.023
Sugar	. . .	0.1	0.3
Ash	. . .	0.225	0.336

Barker and Russell ("Analyst," xxxiv. 125) find the amount of P_2O_5 present to vary between 0.013 per cent to 0.023 per cent.

Grignon gives the following analyses of French ciders ("Le Cidre," Paris, 1887):—

	Sparkling Sweet.	Sweet.	Dry.	Dry.	Old Ciders, annually treated with fresh must.
	Per cent.	Per cent	Per cent	Per cent	Per cent
Alcohol (by volume)	3.8	4.1	5.4	5.4	7.0
Extract . . .	6.41	6.40	3.03	2.95	2.22
Ash . . .	0.29	0.28	0.27	0.26	0.24
Acidity as H_2SO_4 .	0.36	0.39	0.52	0.58	0.54
Sugar . . .	3.47	3.75	0.65	0.58	0.27

The Paris Municipal Laboratory authorities hold that pure cider should contain a *minimum* of 3 per cent of alcohol by volume, 1.8 per cent of extract, and 0.17 per cent of ash, but these low limits are rarely found in practice. Other French authorities are content with 0.9 per cent of extract and 0.12 per cent of mineral matter.

By a decree of 20 July, 1908, cider and perry are legally defined in France as follows:—

"No drink is to be sold (1) under the name of cider unless it is derived exclusively from the fermentation of the juice of fresh apples, or a mixture of fresh apples and pears extracted with or without the addition of water or (2) under the name of perry unless it is derived exclusively from fresh pears with or without the addition of water. The term *cidre pur jus* or *poire pur jus* is reserved for cider or perry obtained without the addition of water. The term *cider* or *perry* is reserved for cider or perry containing at least 3.5 per cent of alcohol .12 gm. per litre of extract (sugar being deducted), and 1.2 gm. of mineral matter per litre. Cider or perry falling below these limits is to be called *petit cidre* or *petit poire*.

The presence of a trace of boric acid in genuine cider (see below) may be used as evidence of its purity, since most artificial ciders are free from boric acid.

Artificial ciders are also usually free from tannin, and the presence of tannin is useful evidence of the authenticity of a given sample.

According to Barker and Russell ("Analyst," xxxiv. 125), if genuine cider be shaken with an equal volume of ethyl acetate for five minutes, and the ethyl acetate separated and poured on to lime water, a band of yellow colour—not persisting long—is developed at the junction of the liquids if pure apple juice be present.

The ash of genuine cider has the following composition :—

	Per cent
Silica	0.94
Phosphoric acid	12.68
Lime	2.77
Magnesia	0.94
Oxide of iron and manganese	0.94
Potash	53.74
Soda	1.10
Carbonic acid	25.78

Pure cider is always lævorotatory. In the presence of added cane-sugar, the rotation will frequently be to the right. If the cider be dextrorotatory, and after inversion is still dextrorotatory, it is certain that commercial glucose is present.

Allen gives the following details for the determination of boric acid in cider.

The *detection* of boric acid in cider and fruits can be readily effected by evaporating 20 c.c. of cider or apple-juice to dryness and igniting the residue, or by directly igniting 25 grms. of apple or other fruit. The ash is rendered distinctly acid to litmus with dilute hydrochloric acid, a piece of turmeric paper partially immersed in the liquid, and the whole evaporated to dryness on the water bath in a flat porcelain dish. The residue is further dried in the water oven for a short time. In the presence of boric acid the turmeric paper will acquire a brownish-red colour, which, on being moistened with a drop of caustic soda, is changed into a variety of colours, chiefly green and purple.

The *quantitative determination* of boric acid in cider and fruits is very troublesome, and this has been the subject of numerous experiments. The difficulty of the analysis is enhanced owing to the minute quantity of boric acid present, and the determination is further complicated by the presence of phosphates. These salts render inapplicable the direct employment of R. T. Thomson's well-known process ("J. S. C. I.," 1893, p. 433), in which the solution is first made neutral to methyl-orange and then titrated with caustic soda and phenol-phthalein in presence of glycerin, the end-point of the titration corresponding to the formation of NaBO_2 . The unsuitability of Thomson's method without modification in the presence of phosphates is due to the fact that while phosphates of the formula MH_2PO_4 are neutral to methyl-orange, they are acid to phenol-phthalein. A number of experiments were made with a view of overcoming the difficulty caused by the presence of phosphates in quantity, but without success. It does not seem possible to make an allowance for the disturbing action of the phosphates, nor does the addition of glycerin after the aqueous liquid has been rendered neutral to phenol-

phthalein overcome the difficulty, owing to the fact that boric acid is distinctly, but indefinitely, acid to phenol-phthalein, even in the absence of glycerin.

After a large number of experiments, the following method for the determination of boric acid in cider, etc., based on the moderate solubility of calcium borate in water, was devised: About 100 c.c. of cider or other liquid is evaporated to dryness with a few cubic centimetres of a 10 per cent solution of calcium chloride; or, in the case of fruits, about 50 grms. weight is cut up into small pieces and the solution of calcium chloride poured over the mass, which is then evaporated to dryness. The dry residue is well charred, boiled with about 150 c.c. of distilled water, and the liquid filtered. The carbonaceous residue is thoroughly incinerated at a moderate temperature, and when cold boiled with a further quantity of 150 c.c. of water, and allowed to stand in the cold for some hours, or preferably overnight. The liquid is then filtered cold, and the filtrate added to the first extract.¹ The mixed aqueous extracts are next evaporated to a volume of 25 or 30 c.c., and after cooling neutralized² by decinormal acid, using methyl-orange as indicator.² An equal volume of glycerin is next added, and the liquid titrated with phenol-phthalein and one-twentieth normal caustic soda solution (free from carbonate). About 10 c.c. more glycerin should now be added, when, if the titration is complete, the red coloration will remain. Each cubic centimetre of the one-twentieth normal solution of caustic soda required represents 0.00175 gm. of boric anhydride, B_2O_3 ; 0.0031 gm. of crystallized boric acid, H_3BO_3 ; or 0.004775 gm. of crystallized borax, $Na_2B_4O_7 + 10H_2O$. The above process gives good results when the amount of boric acid present in the sample taken is not less than 0.005 gm.

Allen has also examined the well-known method for the determination of boric acid based on the volatility of methyl borate, and find the following to be the best method of operating: A suitable quantity of the substance under examination is treated with calcium chloride solution as already described, and well charred, and the main portion of the salts extracted with about 50 c.c. of water. This aqueous extract is transferred to a distillation-flask of about 100 c.c. capacity, and cautiously evaporated nearly to dryness over a naked flame. Meanwhile the charred residue is incinerated, the ash (nearly white) moistened with 2 c.c. of strong sulphuric acid, and the mixture warmed. When the evolution of hydrochloric acid gas is nearly at an end, the acidified residue is transferred to the distilling-flask containing the evaporated aqueous extracts. The last portions are washed in with 10 c.c. of methyl alcohol,³ the flask immersed in a boiling water bath, and the liquid distilled almost to dryness. A

¹ It is desirable to extract the residue for a third time with hot water, allowing the liquid when cold to stand for some time before filtration. This third extract when titrated separately will generally be found to be free from boric acid. If not, the amount found must be added to that already extracted.

² Care should be taken that all the borate is in solution before the titration is begun.

³ Ordinary wood-spirit of good quality, purified by redistillation over caustic potash, is suitable for this purpose.

further addition of 10 c.c. of methyl alcohol is then made, and the distillation repeated. As many as six such treatments are usually required. Between each distillation the residue in the flask should be allowed to cool before the next addition of methyl alcohol is made. The residue finally contained in the distilling-flask should be tested by the flame-reaction with alcohol to ensure that the whole of the boric acid has been volatilized. If this is not found to be the case, the distillation should be repeated once or twice more.

The alcoholic vapours are passed into 25 c.c. of water contained in a flask, the end of the condenser-tube dipping into the liquid. When the process is completed, the distillate is evaporated over a water bath until free from alcohol. By this treatment the methyl borate is hydrolysed, and the boric acid left in a free state. The residual liquid is diluted with a little water and rendered exactly neutral to methyl orange. An equal volume of glycerin is then added, and the liquid titrated with one-twentieth normal caustic soda and phenol-phthalein as already described.

The glycerin used in these processes should be rendered neutral to phenol-phthalein just before use, as it is generally slightly acid in reaction.

In many of the processes already in use for the separation of boric acid by distillation, the methyl borate is distilled into a solution of caustic soda, and after evaporation of the alcohol the aqueous liquid is titrated in the usual way. In Allen's experience, however, when an alkali was used, the results were always above the truth, even when specially purified methyl alcohol was employed. For this reason the use of caustic soda is not to be recommended, and, as previous experiments have shown, is quite unnecessary.

The following results were obtained in a series of experiments made to test the accuracy of the processes here described. A known weight of crystallized borax was added either to a mixture of calcium chloride, magnesium sulphate and sodium phosphate, or to a known weight of apple. In the latter case an exactly similar portion and weight of the same apple was treated with calcium chloride and the boric acid determined, and deducted from that found in the other portion to which borax had been added:—

	No. of Experiment.	Substances Added to the Borax.	Borax Taken.	Borax Found.
Extraction Method.	(1)	Calcium chloride, magnesium sulphate, and sodium phosphate	Gm.	Gm.
	(2)		0.200	0.198
	(3)	50 grms. of apple	0.200	0.204
	(4)		0.020	0.019
Distillation Method.	(5)	None	0.020	0.020
	(6)	None	0.200	0.197
	(7)	Sodium phosphate	0.020	0.022
	(8)	Sodium phosphate	0.200	0.201
			0.020	0.023

Richmond and Harrison's method ("Analyst," xxvii. 179) for the determination of boric acid in butter is rapid and accurate for its intended purpose, but the presence of phosphates in fruits and fruit-products renders the process unsuitable for the determination of boric acid in these substances.

A colorimetric method for the determination of boric acid in milk and other foods has been devised by Cassal and Gerrans ("Brit. Food Journal," October, 1902). The process is based upon the fact that in the presence of oxalic acid the colouring matter of turmeric forms with boric acid an intense magenta-red colour more delicate than the ordinary turmeric reaction (that is, when obtained in the absence of oxalic acid), and permanent for many hours. The alcoholic solution of the colour formed in the reaction is compared with that from a known weight of boric acid. The method is said to be reliable and accurate, but appears to be rather lengthy and tedious.

The following table shows the proportion of boric acid contained in various fruits and ciders, etc., examined:—

Fruits, etc.	Boric Acid, H_3BO_3 .
(1) Apple (Norfolk)	0.009 per cent.
(2) Apple (fox whelp)	0.013 "
(3) Apple (old fox whelp)	0.011 "
(4) Pear, no. 1	0.007 "
(5) Pear, no. 2	0.016 "
(6) Quince	0.016 "
(7) Pomegranate	0.005 "
(8) Grapes	0.004 "
(9) Norfolk cider	0.009 grm. per 100 c.c.
(10) Hereford cider	0.017 " "
(11) Devonshire cider	0.004 " "
(12) Apple juice (Devon)	0.004 " "

Perry, which is the fermented juice of the pear, differs from cider principally in containing less malic acid, and therefore appearing to be more sweet.

Allen gives the following analysis of sparkling perry:—

	Worcestershire.	Devonshire.	Gloucestershire.
	Per cent	Per cent	Per cent
Specific gravity . . .	1.020	1.021	1.070
Alcohol by weight . . .	4.61	4.81	3.64
Solids	6.51	6.49	4.50
Volatile acid as acetic . . .	0.41	0.35	0.22
Fixed acid as malic . . .	0.25	0.20	0.24
Glucose	2.71	3.60	0.36
Sucrose	none	0.31	none
Ash	0.40	0.28	0.30

Truelle gives the following figures for pear juice before fermentation in parts per 1000 :—

	Mean.	Maximum.	Minimum.
Specific gravity	1.0845	1.0980	1.0675
Invert sugar	145.64	200.	108.1
Sucrose	36.74	61.41	16.69
Total fermentable sugar as dextrose	184.14	220.	143.78
Tannin	1.78	3.2	1.01
Pectin	13.08	18.	3.
Acidity as H_2SO_4	1.47	2.40	0.76

The only practical adulterant of cider or perry is the dilution of either the must or the fermented liquor with water. Sometimes preservatives are added, which may be detected as in wine, and occasionally citric or tartaric acid is added to modify the acid flavour.

The preservatives used are salicylic acid, boric acid and sulphites. But as boric acid is a constituent of apples it is probable that in many reported cases it was merely a natural constituent. A boric acid reaction with turmeric can be obtained from 20 grms of pure cider.

The best method of deciding whether water has been added is to calculate the amount of solids present in the original must, as follows :—

$$\begin{array}{rcl}
 \text{Alcohol per cent by weight} & \times 2.07 & = \text{original sugar fermented.} \\
 \text{Acetic acid} & \times 1.5 & = \text{ " " " } \\
 \text{Extractive matter in sample.} & &
 \end{array}$$

The sum of these is equal to the original solids of the juice, and rarely falls below 12 per cent. An excessive amount of solids indicates added saccharine matter.

CHAPTER VI.

FLESH FOODS.

THE inspection of fresh flesh foods does not come within the scope of the present work. Chemical methods are rarely applicable to the examination of fresh flesh from an analytical point of view, and such flesh inspection comes rather within the purview of the meat inspector and the veterinary surgeon than the analyst. The present section is intended to deal more with preserved foods in the sense of flesh foods preserved in tins (or glasses) and in the form of sausages. Further, chemical methods for discriminating between various meats are entirely lacking, and no attempt will be made in this chapter, to so discriminate except so far as the detection of horse-flesh in preserved meat is concerned. The subject will be dealt with from the following points of view.

- (1) The principles underlying the decomposition of flesh food.
- (2) The examination of preserved food from the point of view of
(a) decomposition products, (b) metallic contamination, (c) the presence of preservatives.
- (3) Sausages.
- (4) Meat extracts.

THE DECOMPOSITION OF FLESH.

It has long been known that the products of putrefaction of flesh are ultimately of a very dangerous character. This is especially the case in even the early stages of the decomposition of fish; it is usually only at a later stage that the decomposition products of ordinary meat become dangerous; and at a still later stage, those of the foods embraced by the word "game"—which may be eaten safely when at a decidedly later stage of decomposition than either ordinary meat or fish. Early observers such as Barrows, Kerner, and Panum appear to have recognized in the products of flesh decomposition certain nitrogenous substances, which they believed to be in some way similar to the vegetable alkaloids. But it was Zuelzer and Sonnenschein who first definitely described a flesh-decomposition product as an alkaloid. Later researches have indicated that the principal poisonous decomposition products of flesh are those nitrogenous principles known as animal alkaloids or ptomaines.

The leucomaines are closely allied to the ptomaines and are also known by the name physiological alkaloids. These are formed by

the breaking down of the nitrogenous matter in the living cell and are usually non-toxic. The classification, however, overlaps, as many of these bodies are elaborated by the living cell, as well as formed by the decomposition of dead flesh through the agency of bacteria.

The modern advances in our knowledge of this branch of a most difficult subject are due to Selmi, Nencki, Gautier and Brieger. The first ptomaine to be separated in a state of purity was that isolated by Nencki, and later many were described in detail by Gautier and Brieger. The following summary of the principal ptomaines is due to Gautier and has been adopted by Mitchell ("Flesh Foods").

Monamines of the Fatty Acid Series.

Trimethylamine $(\text{CH}_3)_3\text{N}$. Herring pickle.
 Diethylamine $(\text{C}_2\text{H}_5)_2\text{NH}$. Putrid meat extract.
 Triethylamine $(\text{C}_2\text{H}_5)_3\text{N}$. Decomposed cod-fish.
 Propylamine $(\text{C}_3\text{H}_7)\text{NH}_2$. Decomposing cod-liver.
 Butylamine $(\text{C}_4\text{H}_9)\text{NH}_2$. Decomposing cod-liver.
 Amylamine $(\text{C}_5\text{H}_{11})\text{NH}_2$. Cod-liver oil.

Diamines of the Fatty Acid Series.

Putrescine or Tetramethylene-diamine $\text{C}_4\text{H}_{12}\text{N}_2$. Putrid horse-flesh.
 Cadaverine, or Pentamethylene-diamine $\text{C}_4\text{H}_{14}\text{N}_2$. Putrid fish and blood.
 Neuridine $\text{C}_5\text{H}_{14}\text{N}_2$. Putrid meat, albumin, gelatin.
 Saprine $\text{C}_5\text{H}_{14}\text{N}_2$. Decomposed flesh.

Guanidines.

Methylguanidine $\text{C}_2\text{H}_7\text{N}_3$. Putrid horse-flesh and beef.

Aromatic Ptomaines, free from Oxygen.

Collidine $\text{C}_8\text{H}_{11}\text{N}$. Putrid fish and putrid gelatin.
 Parvoline $\text{C}_9\text{H}_{13}\text{N}$. Putrid horse-flesh after several months.
 Corindine $\text{C}_{10}\text{H}_{13}\text{N}$. Putrid cuttle-fish.
 Dihydrocollidine $\text{C}_8\text{H}_{13}\text{N}$. Putrid fish and horse-flesh.

Oxygenated Ptomaines.

Neurine $\text{C}_5\text{H}_{13}\text{NO}$. Putrid meat on fifth or sixth day.
 Choline $\text{C}_5\text{H}_{15}\text{NO}_2$. Accompanies neurine.
 Muscarine $\text{C}_5\text{H}_{15}\text{NO}_3$. Putrid fish.
 Betaine $\text{C}_5\text{H}_{11}\text{NO}_2$. In mussels (leucomaine).
 Homopiperidinic Acid $\text{C}_5\text{H}_{11}\text{NO}_2$. Decomposition of meat fibrin.
 Mytilotoxine $\text{C}_6\text{H}_{15}\text{NO}_2$. In poisonous mussels (? leucomaine).
 Mydatoxine $\text{C}_6\text{H}_{13}\text{NO}_2$. Putrid horse-flesh after nine to fifteen months.
 Gadinene $\text{C}_7\text{H}_{17}\text{NO}_2$
 Methylgadinene $\text{C}_8\text{H}_{19}\text{NO}_2$ } Putrid fish, especially cod.
 Unnamed base of Brieger $\text{C}_7\text{H}_{17}\text{NO}_2$. Accompanies mydatoxine

Aromatic Oxygenated Bases.

Tyrosamines C_7H_9NO ; $C_8H_{11}NO$; $C_9H_{13}NO$. Decomposing cod-liver.

Mydine $C_8H_{11}NO$. Decomposing human flesh.

It may be mentioned that Brieger considers that these poisonous bases which Gautier claims to be included in the group of the leucomaines, are not in reality the products of the cell metabolism, but are in fact absorbed into the cell from the intestines.

The question of ptomaines in regard to toxicological analysis will not be discussed, but it may not be out of place to mention that there are many ptomaines, closely resembling well-known vegetable alkaloids in their reactions, especially their mydriatic effects. Zuelzer and Sonnenschein some years ago isolated a septic alkaloid which resembled atropine and hyosciamine in a very remarkable manner.

A brief reference to the symptoms of ptomaine poisoning may now be made.

Symptoms of Ptomaine Poisoning.—The usual symptoms of ptomaine poisoning are as follows: A dilated then contracted pupil of the eye, feeble respiration, weak pulse, temperature sub-normal, skin moist, loss of the power of contracting the muscles, stupor, convulsions, and death. The loss of muscular contractibility takes place even when under the influence of electricity, and is one of the determining features of poisoning by muscarine, a ptomaine found in putrefying fish and in poisonous mushrooms.

The action of ptomaines on the body varies considerably; some have little effect whilst others are fatal in even small quantities. It is not unlikely that the symptoms of flesh poisoning vary in nature and extent according to the kind and amount of the bases present, some of which probably modify in a greater or less degree the action of the others.

The methylamines and ethylamines formed during the putrefaction of flesh are the only monamines not very poisonous; large quantities of butylamine produces convulsions and muscular paralysis; and amylamine which is extremely poisonous causes the pupils of the eye to dilate and, finally, convulsions. The diamines (putrescine, cadaverine, neuridine and saprine) have very little or no effect on the body, and are only considered slightly poisonous. Cadaverine may produce inflammation of the mucous membrane.

Methylguanidine, which may be considered the representative guanidine ptomaine, is extremely poisonous. When it is injected into a small animal it causes dilation of the pupils, convulsions, and death within twenty minutes.

Of the aromatic non-oxygenated ptomaines, collidine, parvoline, corindine and dihydrocollidine are all exceedingly poisonous. Corindine, like curare, produces paralysis. Dihydrocollidine produces torpor, muscular paralysis, and convulsions.

Of the better-known oxygenated ptomaines neurine causes an excessive flow of saliva, contraction of the pupils, sudden convulsions, and death.

Choline acts physiologically much in the same way as neurine, but not so violently.

Muscarine is very poisonous, and small doses will produce salivation, contraction of the pupils, diarrhœa, convulsions, and death. Atropine is used as an antidote, as its action is opposite to that of the three foregoing ptomaines. Betaine is non-poisonous. Mydatoxine is somewhat poisonous. Large doses cause diarrhœa, redness of the eyes, convulsions, and death. Gadinene is not very poisonous, though methylgadinene in large doses produces symptoms of paralysis. An unnamed base of Brieger ($C_7H_{17}NO_2$), found with mydatoxine in putrid horse-flesh, resembles curare in its poisonous properties.

Botulism or Sausage Poisoning.—Cases of botulism, like the attacks of trichinosis, have been most prevalent in those parts of Germany, Saxony, for example, where raw ham and raw sausage are largely consumed. There have been wholesale cases of poisoning as at Chemnitz in 1879, when 241 persons were poisoned by Mettwurst, and 160 met the same fate seven years later. Ostertag mentions similar but smaller outbreaks since 1886, as for example in Dresden (11), in Gerbstadt (over 50), and in Gera (30).

The distinguishing symptoms of pure botulism can be detected after a period of incubation of from eighteen to forty-eight hours. They are an uneasy and heavy feeling in the stomach accompanied by vomiting and sometimes diarrhœa, faintness, blurred vision, flaccidity of the muscles and collapse. If the case is fatal, death ensues in from four to eight days. If the toxine of *B. botulinus* is the sole cause of the illness, neither fever nor mental disturbances occur as symptoms. According to Senkpiehl out of 412 cases recorded between 1789 and 1886, 165 proved fatal, thus the mortality is very high.

Eber considered both sausage poisons and ptomaines as toxigenic substances, and not as toxines. He grouped together under the term "toxigenes" those chemical products which, when injected into an animal, are not poisonous until modified by the vital activity of the cells. He compared them with sodium iodide and similar inorganic substances which, when injected into an animal, produce no ill effects for at least six or eight hours. For years the origin of the poison could not be determined, though it was well known to be distinct from that produced by ordinary putrefaction. Hilger was the first to obtain from the intestines of six persons who had died from sausage poisoning, a semi-fluid substance which closely resembled curare. Tamba also found a similar substance in liver sausage which had been exposed to the air. Haupt concluded that the decomposition products formed by *B. proteus mirabilis* caused the disease. Ostertag, however, showed that the symptoms of botulism were not identical with those produced by the inoculation of cultivations of that micro-organism. In 1895 van Ermengem extracted an anaërobic bacillus from the body of a person who had died from sausage poisoning, and the cultivations of this produced the same symptoms.

Brieger and Kempner have recently extracted a toxine from a pure cultivation of *B. botulinus*, which they consider closely related to the toxines of diphtheria and tetanus in chemical composition.

The isolation of ptomaines in preserved meat would, in general, be very powerful evidence of the decomposition of the food which would probably have caused more or less severe illness.

The most important of the earlier methods devised for the separation of the ptomaines is that used by Brieger, who is perhaps the greatest authority on this subject. He uses the salts of the heavy metals, and picric acid. For the purpose of separating an alkaloid from a putrefying mass, this mass is first boiled with acidified water, and then filtered; the filtrate is treated with subacetate of lead; from this, excess of lead is precipitated by sulphuretted hydrogen which is passed through the filtrate, and the fluid is again filtered to keep back the lead sulphide. This second filtrate is evaporated to about one-third of its original bulk, and is washed with amyl alcohol to remove fat, etc., and again reduced in bulk by evaporation, and sulphuric acid and ether added; the ether is removed, after which the remaining liquid is concentrated by careful evaporation to one-fourth of its bulk; the evaporation drives off most of the volatile fatty acids present, after which the fluid, neutralized by the addition of baryta, is again filtered, carbonic acid gas is passed through it, by which barium carbonate is thrown down, which is separated by filtration. After careful heating over a water bath, the fluid is cooled, and bichloride of mercury is added, when a somewhat dense precipitate is formed. This precipitate is carefully washed and decomposed by sulphuretted hydrogen, when sulphide of mercury is thrown down; the fluid is again filtered and the filtrate is evaporated to obtain as great concentration as possible. From the liquid so obtained all inorganic substances crystallize out first; these are removed, and then in the fluid that remains "organic" acicular crystals are thrown down. These may be dissolved in water, but they are insoluble in absolute alcohol, ether, benzene, or chloroform. It is found that these substances, the ptomaines, may be precipitated by the salts (especially the chlorides) of the heavy metals. These precipitates or crystals differ, however, very considerably as to their solubility; hydrochloride of putrescine obtained by the above method separates out in acicular crystals, and on the addition of chloride of gold gives very insoluble crystals of an octahedral form, whilst on the addition of chloride of platinum, octahedral crystals, which are much more soluble, are also formed. Phospho-molybdic and phospho-tungstic acid added to this substance give respectively a yellow and a white crystalline precipitate. Iodide of mercury dissolved in iodide of potassium also gives rise to the formation of prisms; with ferrocyanide of potassium there is a yellowish amorphous precipitate; with picric acid a yellow precipitate composed of delicate needle-shaped crystals; and with an aqueous solution of bichloride of mercury an exceedingly insoluble acicular crystalline precipitate is thrown down. This substance and the reactions obtained with it may be taken as typical of the whole group, although there are certain differences; for instance, cadaverine treated with chloride of gold gives a very soluble substance, whilst with chloride of platinum there are thrown down well-formed very insoluble crystals. Mydaine is exceedingly soluble in most of its combinations, and it is at present

almost impossible to separate it from the mother liquid; in fact, its salts have not yet been separated, and in consequence it has been found impossible to determine its exact chemical nature. These, along with saprine, were obtained by Brieger from flesh that was being decomposed by the action of putrefactive micro-organisms.

Brieger's later method consists in extracting the finely divided substances with very dilute hydrochloric acid, evaporating the extract on a water bath, filtering and finally concentrating to the consistency of a syrup. This is dissolved in 90 per cent alcohol and the liquid filtered and excess of an alcoholic solution of HgCl_2 added. The whole is allowed to stand for twenty-four hours when the precipitate is collected and washed with water, then suspended in water and decomposed by a current of H_2S . The precipitated sulphide of mercury is removed by filtration and the ptomaines are now present as hydrochlorides in the filtrate, after which they are examined as in the former method.

The Stas-Gautier method is as follows:—

To the finely divided substance add water containing 0.5 per cent of tartaric acid and allow to digest for twenty-four hours. Filter the liquid and separate the last portions by pressure. If the substance submitted for examination is liquid or almost liquid, slightly acidify with tartaric acid; if it is oily, shake in a flask containing carbon dioxide, with an aqueous 0.25 per cent solution of oxalic acid.

Heat the slightly acid extract, or acidified original liquid, for a moment at 100°C . to coagulate albuminous substances, then cool and filter. Evaporate the filtrate in a vacuum at 40°C . to a syrup, collecting the distillate. This is extract A.

The distillate generally contains substances carried over with the water, as phenols, indol, volatile fatty acids, ammonia, substituted ammonias, etc., with traces of volatile ptomaines. To recover the latter, acidify with a slight excess of sulphuric acid, and, to free the bases and get rid of the larger proportion of the ammonia they contain, treat the dried sulphates with lime. Shake the mixture of calcium sulphate and free bases with ether, then with alcohol. Any calcium oxide which dissolves can be precipitated with a very small quantity of sulphuric acid, leaving the bases in solution.

To remove fatty substances, lactic acids, excess of acid added, etc., from extract A, extract with ether then add boiling alcohol, which gives solution B and leaves residue C.

Take up with water and dialyse residue C, which contains salts, extractives, xanthic bodies, acid amides, etc. Concentrate the part passing through, by evaporation, precipitate the bases present with lead acetate, remove the lead by adding hydrogen sulphide, concentrate the filtrate and add alcohol. The substances gradually accumulating consist of oxygenated bases, such as leucine.

Evaporate solution B, which contains the most important bases, with peptones, etc., to a syrup. Make alkaline with potassium bicarbonate, mix with powdered glass, and extract first with ether, then chloroform, and lastly amyl alcohol.

The first two extracts after being evaporated leave a residue of any

alkaloidal substances extracted. Shake the amyl alcohol with water to which has been added a little sulphuric acid, thus extracting the bases in solution. Boil the liquid and add a hot solution of barium hydroxide so long as a precipitate forms. Separate the bases, which are left in solution, into fixed and volatile bases, by distillation, and allow the distillate to pass into acidulated water.

Dragendorff has devised the following process:—

Dragendorff's Method.—Mix the finely divided substance with water acidified with a little sulphuric acid, digest for several hours at 50° C., then wash with water. Evaporate the liquid to a syrup, then digest for twenty-four hours with three or four times its volume of 95 per cent alcohol. Filter off the separated substances, evaporate the alcohol from the filtrate, shake the aqueous residue with benzene to remove certain impurities. Make the residue alkaline with ammonia and again extract with benzene, which this time removes some free bases. Acidify the liquid and extract with chloroform, again make alkaline with ammonia or sodium carbonate, and again extract with the same solvent. Extractions are made in a similar manner with amyl alcohol, first from acid and then from alkaline solution. Finally recover and examine the bases from each of the various extracts.

The following summary of the properties of the principal of the ptomaines is based on the work of Brieger and Gautier, and is due to C. A. Mitchell:—

(A.) AMINES OF THE FATTY SERIES.

(1) MONAMINES.

Trimethylamine (CH_3)₃N. Met with in herring pickle, ergot of rye, and in putrefaction products of meat, cheese, etc. Is a gas with fish-like odour. Boils at 9.3° , solidifies at about -75° . Very soluble in water, forms well-marked salts; the aurochloride forms yellow monoclinic prisms and the platinochloride orange prisms.

Ethylamine (C_2H_5)₂NH₂. Met with in putrefying flour. Strongly alkaline liquid, boiling at 18.7° with an ammoniacal odour.

Diethylamine (C_2H_5)₂NH. In decomposing fish, meat extracts, or sausages. A volatile, inflammable liquid, boiling at 57.5° . Very soluble in water. It can be separated from ethylamine by treating the mixed mercurio-chlorides with acetic acid, in which the diethylamine salt is insoluble.

Triethylamine (C_2H_5)₃N. Accompanies the two last described and other bases in decomposing fish or peptones. Strongly alkaline, inflammable, liquid boiling at 89° . Is slightly soluble in water, and is precipitated from its solution by salts of mercury, copper, lead, or iron. The aurochloride soon darkens by reduction to amino chloride.

Propylamine (C_3H_7)NH₂.—Is found in decomposing gelatine and cod-liver. Is an alkaline liquid, boiling at 78° to 82° , soluble in water. Its platinochloride forms monoclinic prisms.

Isopropylamine (C_3H_7)NH₂.—Is an ammoniacal liquid, boiling at

32°. It is soluble in water. Its platinochloride forms orange plates.

Butylamine (C_4H_9) NH_2 . In decomposing cod-liver. Is an alkaline liquid boiling at 76°. Its solution reduces copper and silver salts on warming. Its platinochloride forms yellow plates fairly soluble in water.

Isoamylamine (C_5H_{11}) NH_2 .—In decomposing cod-liver. A colourless liquid of disagreeable odour, of specific gravity 0.797.

Herylamine (C_6H_{13}) NH_2 .—In decomposing cod-liver and putrefying yeast. A liquid boiling at 129°. It forms a hydrochloride in crystalline lamellæ, and a platinochloride in orange scales.

(2) DIAMINES.

Ethylidene diamine (C_2H_4) $(NH_2)_2$.—Properties doubtful. To be found in putrefying fish. Is probably identical with ethylene diamine. An alkaline liquid boiling at 116°.

Tetramethylene Diamine (CH_2 . CH_2 . NH_2) $_2$ or *Putrescine*. Is found in the putrefactive products of flesh. Neuridine appears to be formed first (q.v.), to be replaced by cadaverine and putrescine. Putrescine is a clear mobile liquid with a strong characteristic odour. It rapidly absorbs CO_2 from the air, forming a crystalline carbonate, boils at 158° when quite pure, and melts at 24°. It forms a crystalline hydrochloride in long transparent needles, crystallizable from hot dilute alcohol. Brieger gives the following summary of the reactions of the free base:—

Phosphotungstic acid	= white ppt., soluble in excess.
Phosphomolybdic acid	= yellow ppt.
Potassio-mercuric iodide	= oily ppt., afterwards becoming crystalline.
Potassio-bismuth iodide	= " " "
Potassio-cadmium iodide	= " " "
Picric acid	= yellow needles.
Tannic acid	= dirty white ppt.

The following method of separation from neuridine and cadaverine may be adopted. The solution is precipitated with platinum chloride, and the separating platinochlorides are treated with excess of cold water. The putrescine salt is very insoluble, and on filtration is left with some cadaverine salt. On heating the precipitate diluted with more water, the putrescine and cadaverine salts dissolve, and the putrescine salt separates out first on cooling.

Pentamethylamine Diamine (CH_3) $_5(NH_2)_2$. Cadaverine. It appears after about the third day of putrefaction of flesh. Is often associated with neuridine and putrescine. It is a viscid liquid, boiling at 178° and rapidly absorbing CO_2 from the air. It has a penetrating odour. It yields the following reactions when in solution in water:—

Phosphotungstic acid	= white ppt., soluble in excess.
Phosphomolybdic acid	= " " " "
Potassio-mercury iodide	= resinous ppt.
Potassio-cadmium iodide	= " " gradually becoming granular.
Iodine in potassium iodide	= brown ppt.
Potassio-bismuth iodide	= " " "
Picric acid	= yellow needles.
Tannic acid	= white amorphous ppt.
Potassium ferricyanide and ferric chloride	= blue coloration.

It can be separated from putrescine and neuridine by precipitating the hydrochlorides with a solution of platinum chloride. By fractional crystallization the platinochlorides of cadaverine and putrescine separate first, the more soluble salt of neuridine being left in the mother liquor. The crystals separating are suspended in water and decomposed by a current of H_2S . By filtering off the platinum sulphide, the hydrochlorides are left, which are obtained by evaporating the solution, and by treatment of the residues with 96 per cent alcohol at a temperature of 60° to 70° ; the cadaverine hydrochloride is dissolved and the putrescine salt left insoluble.

Neuridine $C_7H_{14}N_2$. Is formed in the putrefactive decomposition of meat, fish, albumin, or gelatine, and reaches its maximum on the eleventh or twelfth day. Brieger separates it in the following manner. The finely divided mass is extracted with hot water slightly acidified with HCl , the extract filtered, the filtrate concentrated to a syrupy liquid on the water-bath, and this repeatedly extracted with alcohol. The alcoholic filtrate is treated with mercuric chloride solution, the precipitate collected and washed and then decomposed in suspension by a current of H_2S . The liquid is filtered, and concentrated on the water bath, and on cooling long needle-shaped crystals of neuridine hydrochloride separate out, which can be purified by recrystallization from hot dilute alcohol. Free neuridine is insoluble in alcohol or ether, but is soluble in water. It gives the following reactions:—

With phosphotungstic acid	= white amorphous ppt., soluble in excess.
„ phosphomolybdic acid	= „ crystalline ppt.
„ picric acid	= ppt. appears slowly, and becomes yellow needles.
„ potassio-bismuth iodide	= red amorphous ppt.
„ gold chloride	= crystalline ppt.

Saprine $C_8H_{16}N_2$. Occurs in decomposing flesh. Not very poisonous.

(3) GUANIDINE DERIVATIVES.

Methyl-guanidine, $NH : C(NH_2) (NH.CH_3)$. Occurs in decomposed flesh products. The free base is crystalline and deliquescent. It forms a crystalline hydrochloride, insoluble in alcohol. The platinochloride forms rhombic crystals, readily soluble in ether. Is very poisonous.

(4) AROMATIC AMINES.

Pyridine C_5H_5N . Found in the decomposition products of proteids. Liquid of penetrating odour, miscible with water. Boils at 114° .

Collidine $C_8H_{11}N$. Found in putrid fish. A yellow liquid of acrid odour. Slightly soluble in water. Specific gravity 0.986; boiling point 168° . Is very poisonous.

Parvoine $C_9H_{13}N$. From putrid horse-flesh. Amber-coloured oil, boiling above 200° . Slightly soluble in water.

Corindine $C_{10}H_{15}N$. From putrid fish. A yellow viscous liquid boiling at about 230° . Is poisonous.

Dihydrocollidine $C_8H_{13}N$. In putrid meat and fish. Boils at about 208° . Specific gravity at $0^{\circ} = 1.0296$. Forms a crystalline hydrochloride. Is very poisonous.

(5) OXYGENATED BASES.

Neurine $C_5H_{13}NO$. In putrid flesh, appearing about the fifth or sixth day. Is a syrupy liquid, of strong alkaline reaction. Soluble in water. Forms a crystalline hydrochloride. Is very poisonous. It gives the following reactions:—

- With phosphomolybdic acid = white crystalline ppt., soluble in excess.
- „ phosphotungstic acid = nil.
- „ potassio-mercury iodide = voluminous yellowish-white ppt.
- „ potassio-bismuth iodide = amorphous red ppt.

Muscarine $C_5H_{15}NO_3$. In putrid fish. Is one of the most poisonous ptomaines known. It is separated by Brieger as follows:—

The alcoholic extract of the putrid mass is treated with mercuric chloride to separate choline and neurine. The filtrate is treated with H_2S to remove mercury, and the filtrate concentrated after being neutralized with sodium hydroxide. The syrupy liquid is taken up in alcohol and excess of platinum chloride added. The platinochloride of neuridine crystallizes out first and is filtered off. The filtrate is concentrated, and a fresh crop of crystals (ethylendiamine platinochloride?) is filtered off. On further concentration, the platinochloride of muscarine separates, and this, treated with H_2S , yields the hydrochloride, which is converted into the sulphate by treatment with silver sulphate, and this into the free base by treatment with barium hydroxide. Muscarine forms colourless, deliquescent crystals, which are alkaline, and rapidly absorbs CO_2 from the air.

Mytilotoxine $C_6H_{15}NO_2$. From poisonous mussels. Its aurochloride melts at 182° .

Gadinene $C_9H_{17}NO_2$. From putrid fish. Its platinochloride melts at 214° .

Mydaleine.—Found in decomposing human flesh. Composition uncertain. Is poisonous.

Mydine $C_8H_{11}NO$. In putrid flesh. Picrate melts at 195° . Is not very poisonous.

Tyrottoxine $C_6H_5N_2OH$. From decomposing cheese, and in ice cream. Fine needles melting with decomposition at 90° , in the presence of moisture. Is poisonous.

Apart from the exceedingly difficult question of the isolation of ptomaines, there is another problem connected with the question of preserved meats which presents difficulties which are generally insuperable. This is the question of deciding what meats are actually present in a given sample. It is comparatively rare that this examination is necessary, since high-grade makers keep their preparations true to description, using the necessary palatable admixtures only. But in low-grade preparations, the main constituent is frequently

not true to name, but as no legal standards for this type of preparation exist, it is very difficult to bring cases dealing with the composition of preserved meats, etc., into court, except on the grounds of containing preservatives or being unfit for human food.

Warden and Bose have published ("Chem. News," 1890, LXI. 304) some particularly complete analyses of typical samples of canned beef and mutton. They estimated the moisture to vary from 49 per cent to 57 per cent, the fat from 10 to 22; the porteids (i.e. $N \times 6.25$) from 24.5 to 29; the ash from 0.62 to 4.36; the chlorine from 0.11 to 2.65; the phosphoric acid from 0.31 to 0.40; the hot water extract from 5.35 per cent to 10.14 per cent, with a content of nitrogen varying from 0.88 per cent to 1.10 per cent.

The following methods of analysis are satisfactory:—

Thoroughly pulp the entire contents of a can in a large mortar, taking care to scrape out any fat and jelly that may be left in the can. Warden considers it a mistake to regard a slice of the contents as a fair sample.

To determine the moisture, place from 5 grms. to 6 grms. of the sample with forceps in a flat platinum dish, and dry first at 100° , then at 120° . Moisten the samples with alcohol then dry again. The whole time of heating takes from eight to nine hours. In another large platinum dish heat from 30 grms. to 40 grms. of the pulp in the manner just described; reduce to a fine powder, and again heat. This dried pulp, preserved in a closely stoppered bottle, can be used for the determining fat, nitrogen, and aqueous extract.

To determine the ash, char that portion of the pulp used for ascertaining the moisture, at a temperature below redness, crush with a glass rod, exhaust with boiling water and again ignite. Treat the residue with boiling water again and ignite and weigh the insoluble ash. Evaporate the aqueous extract to dryness, heat the residue almost to redness, and weigh the soluble ash. The total ash is estimated as the sum of the soluble and insoluble ashes determined as just described, and it will be found that the figures thus obtained coincide with determinations of the total ash by direct ignition, avoiding at the same time the difficulty experienced in the latter case of bringing about complete combustion of the carbon without losing any of the alkali-metal salts by volatilization.

The soluble ash is used for determining potassium and sodium by dissolving it in water, then adding to the warm solution barium chloride ferric chloride, and ammonia successively. The last reagent is used in such quantity as to make the liquid just alkaline. Filter off the precipitate which consists of $BaSO_4$, $FePO_4$, and FeH_3O_3 ; treat the filtrate with ammonium carbonate and ammonium oxalate and warm on the water bath for some time. Remove the precipitate, consisting of $BaCO_3$ and CaC_2O_4 by filtration; evaporate the filtrate in platinum to dryness and gently ignite the residue; re-dissolve the residue in water, filter the solution from a little barium carbonate, add a drop of hydrochloric acid to the filtered liquid and evaporate with platinic chloride to separate the potassium and sodium.

Warden and Bose, to determine the chlorine and phosphoric acid,

employ the following method: Mix 20 grms. of the freshly pulped meat with about two grms. of pure sodium carbonate dissolved in sufficient water to cover the pulp. Evaporate the resulting magma to dryness, carbonize, extract first with water, then with nitric acid, again ignite the residue, dissolve in nitric acid, and determine the chlorine and phosphates in the mixed solutions by the usual methods.

The total nitrogen in the dried pulp is determined by Kjeldahl's process and multiplied by the factor 6.25 to find the proteids.

The extractive matter is determined by boiling 1 gm. of the dry pulp with distilled water in a 100 c.c. flask and when cold diluting to 100 c.c. Pass the liquid through a dry filter and evaporate to dryness an aliquot portion of the very faintly opalescent filtrate in a platinum dish; weigh the residue. The greater part of the filtrate is used for determining extractive nitrogen by Kjeldahl's method.

To determine fat, take 0.5 gm. of the dried pulp in a small well-stoppered weighing bottle, add a measured volume of light petroleum ether from a burette. Allow the mixture to stand for two days, occasionally shaking it, then draw off by a small pipette a portion of the perfectly clear liquid floating on the top, carefully measure a small volume of it and pass into a small beaker. Distil off the petroleum ether, dry the residual fat at 100° and weigh. From this, calculate the fat in the total amount of petroleum ether used. This method of Dragendorff's was found by Warden and Bose to have similar results to those produced by exhausting the substance with a solvent of fat in the customary manner. Warden and Bose have compared their analyses of canned meats examined by the foregoing methods with the figures arrived at by König in the analysis of fresh beef and mutton. They find that whereas the percentage of moisture in canned meat is less than in fresh meat, the fat in canned meat usually exceeds that of fresh meat. They obtain the following amounts of albuminous matters in the anhydrous and fat-free samples examined by multiplying the total nitrogen by 6.25.

	Albuminous Matters in Anhydrous Fat-free Meat,
Average of canned beef samples	87.06 per cent
Average of canned mutton samples	87.19 " "
Average of all fresh cow and ox flesh	93.94 " "
Averages of all fresh mutton	93.81 " "
Average of all canned meat samples	87.12 " "
Average of all fresh meat samples	93.87 " "

König's analyses of seven specimens of canned meats showed them to have the following average composition:—

Proteids, etc., 28.97; fat, 12.63; ash, 3.71; and water, 54.69 per cent. These figures correspond to 10.33 per cent of nitrogen and 27.27 per cent of fat in the anhydrous samples, and to 88.63 per cent of albuminous matters in the anhydrous and fat-free samples.

The following table of analyses of preserved foods is taken from Bulletin 13, part 10, of the United States Department of Agriculture Bureau of Chemistry:—

	Number of Analyses.	Water.	Fat.	Total.	Nitrogen.			Nitrogenous Substances.				Total Ash.	Sodium Chloride.
					Insoluble in Hot Water.	Precipitated by Bromine.	Meat Bases.	Protein N x 6.25.	Proteins Insoluble in Hot Water.	Gelatinoids and Proteins Ppt'd by Bromine.	Meat Bases.		
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Fresh beef	5	68.19	12.60	3.02	2.41	0.19	0.43	18.89	15.08	1.21	1.29	0.96	0.08
		71.17	15.33	3.19	2.52	0.24	0.53	19.94	15.75	1.50	1.65	1.13	0.24
	—	65.81	9.89	2.83	2.32	0.14	0.33	17.69	14.50	0.87	1.02	0.78	trace
Canned beef, roast and boiled													
	22	58.89	13.99	4.15	3.09	0.57	0.49	25.95	19.29	3.59	1.58	1.28	0.53
		66.39	31.78	5.51	4.47	1.16	1.03	34.44	27.94	7.25	3.21	3.51	2.51
Canned beef, corned	—	45.35	5.89	3.31	1.98	0.24	0.09	20.69	12.38	1.50	0.62	0.65	0.04
	11	56.35	11.43	4.26	3.58	0.32	0.36	26.63	22.37	2.02	1.11	4.69	3.37
		60.10	22.68	4.83	4.33	0.53	0.47	30.36	27.06	3.31	1.47	7.38	4.68
Canned beef, smoked and dried	—	49.94	6.33	3.72	3.01	0.20	0.20	23.25	18.81	1.25	0.62	3.57	2.56
	11	47.42	7.46	5.21	4.22	0.15	0.85	32.59	26.41	0.92	2.63	12.51	9.67
		59.58	12.37	6.49	4.91	0.37	1.29	40.56	30.69	2.31	4.02	17.30	11.33
Fresh horse meat	—	39.22	4.59	4.00	3.30	0.06	0.59	25.00	20.62	0.38	1.59	9.58	7.15
	16	69.81	9.61	3.11	2.37	0.20	0.55	19.47	14.83	1.23	1.70	1.01	0.01
		76.91	33.66	3.60	2.97	0.36	1.22	22.50	18.56	2.25	3.81	1.27	0.09
	—	52.16	1.24	2.13	1.33	0.12	0.15	13.31	8.31	0.75	0.47	0.63	trace

	Number of Analyses.	Water.	Fat.	Total.	Nitrogen.			Nitrogenous Substances.				Total Ash.	Sodium Chloride.
					Insoluble in Hot Water.	Precipitated by Bromine.	Meat Bases.	Protein Insoluble in N x 6.25.	Proteins Insoluble in Hot Water.	Gelatinoids and Proteins Ppt'd by Bromine.	Meat Bases.		
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Canned ham and bacon													
Average . . .	13	36.77	37.81	3.11	2.38	0.20	0.54	19.43	14.86	1.22	1.67	5.88	5.84
Maximum . . .		53.30	64.07	6.68	4.75	0.74	1.38	41.75	29.69	4.62	4.31	19.90	17.84
Minimum . . .		15.34	16.84	1.07	0.91	0.06	0.05	6.69	5.69	0.38	0.16	0.78	trace
Canned tongue													
Average . . .	17	55.17	20.23	3.11	2.50	0.21	0.39	19.43	15.64	1.33	1.23	3.71	2.90
Maximum . . .		71.80	38.09	3.95	3.20	0.48	0.72	24.69	20.00	3.00	2.25	6.22	4.42
Minimum . . .		39.58	6.84	2.09	1.76	0.02	0.07	13.06	11.00	0.12	0.22	0.78	0.37
Canned fowl													
Average . . .	10	60.42	16.30	3.53	3.05	0.22	0.26	22.07	19.06	1.39	0.81	1.09	0.05
Maximum . . .		69.06	39.31	5.14	4.45	0.76	0.46	32.12	27.81	4.75	1.44	2.61	0.13
Minimum . . .		47.46	1.82	2.14	1.77	0.11	0.06	13.37	11.06	—	—	0.58	trace
Canned fowl and game													
Average . . .	15	62.44	12.72	3.62	2.86	0.26	0.44	22.61	18.39	1.66	1.39	2.31	1.16
Maximum . . .		70.60	30.97	5.10	4.35	0.39	0.65	31.87	27.19	2.44	2.03	3.82	2.23
Minimum . . .		44.95	2.02	1.60	1.20	0.12	0.18	10.00	7.50	0.75	0.56	1.00	0.37
Canned sausages													
Average . . .	25	58.51	21.82	2.23	1.82	0.19	0.21	13.92	11.37	1.21	0.67	2.86	1.02
Maximum . . .		88.61	41.34	3.86	3.86	0.62	0.42	24.12	20.75	3.88	1.31	6.37	4.68
Minimum . . .		44.02	3.53	0.52	0.38	0.03	0.04	3.25	2.38	0.19	0.12	1.35	0.04

Properly prepared preserved foods in cans or glasses should be hermetically sealed and sterilized by heat. They will then keep for an indefinite time.

If not properly sterilized, gradual decomposition will take place—unless preservatives have been added—and gases due to fermentation will be given off. Any tin that is bulged by internal pressure should be rejected at once. In the trade such tins are known as “blown tins”. If it be necessary to examine the gases given off from such a tin, the process of collecting them described by Doremus (“*Jour. Amer. Chem. Soc.*” 1897, **19**, 730) may be used. The tin, held in place by a clamp, is pierced by a hollow steel needle passing through a closely fitting hole in a rubber cork, which, being pressed hard into the tin forms a tight joint; the needle is connected by a fine glass tube—almost capillary—to an eudiometer or nitrometer tube filled with mercury, and the gas, which may amount to 50 to 80 c.c. in large tins that have decomposed is collected in the ordinary manner and examined. A drop of lead acetate solution will indicate the presence of sulphuretted hydrogen, but the bulk of the gas will usually be found to be CO_2 . Sometimes, however, hydrogen predominates. It must not be inferred that because a slight discoloration is found on the metal of the can that putrefaction has taken place. In a case recently exhaustively investigated by the writer, in which black patches were found, it was proved that the gelatine used to “set” tongues in contained an appreciable amount of sulphurous acid (used to bleach it).

The solder and the surface of the tin were of different metallic composition and appear to have set up electrical action, and the sulphurous acid was partially reduced to H_2S .

No bad odour should be detected on opening the tin; but it must be remembered that, especially with smoked products, there may be a trace of volatile matter derived from the smoking process, which is smelt directly the tin is opened, but which in a few minutes has disappeared. If a piece of red litmus paper held close over the tin which is stood in hot water, turns blue, the contents should be rejected. At the same time a slight alkaline reaction of the actual contents is not evidence of decomposition, as, for example, normal tinned lobsters are often alkaline, but an alkaline reaction should be regarded with suspicion. A microscopic examination is necessary, as, if the meat is in good condition, the muscular fibres will show their characteristic cross striations, whereas if numerous bacteria are found, they often give little coloured patches which destroy these striations in places. Such a sample should be rejected.

The question of metallic contamination is one of considerable importance. In general, one has to be prepared for the presence of tin in tinned goods; lead in such goods when a lead solder has been used; and copper under the following circumstances: (1) where copper utensils have been used for cooking; (2) where much gelatine has been used, and the gelatine is contaminated with copper in the course of its manufacture; (3) where it has been added as copper sulphate to preserve the colour of green vegetables; this, however, being rare in the case of mixed meat and vegetables, but common in the case of

preserved peas and spinach, which should always be examined for copper.

Tin.—In a recent report issued by the Local Government Board, Schryver details the following method for the determination of tin in tinned meats :—

He destroys the organic matter as far as possible by heating with sulphuric acid and potassium sulphate in the manner used in the Kjeldahl process. The clear liquid remaining in the flask is diluted to 100 c.c., and the tin precipitated as sulphide by sulphuretted hydrogen, and filtered after standing overnight. If the quantity of tin present is comparatively large (over 2 grains per lb.), 50 grms. of the sample are treated as above (in two flasks), and the tin converted into oxide and weighed. For smaller amounts of tin, Dr. Schryver devised the following colorimetric process.

The filter paper with the precipitate of tin sulphide, sulphur, etc., from 10 grms. of foodstuff is transferred to a test tube and boiled with 5 c.c. of concentrated hydrochloric acid. The liquid is filtered on a suction filter, the filter being washed with another $2\frac{1}{2}$ c.c. of acid. The air is replaced above the filtrate by a current of carbon dioxide, and a standard strip of zinc foil ($2 \times \frac{1}{2}$ in.) added to the hot liquid. Two c.c. of the special reagent (0.2 gm. of dinitro-diphenylamine-sulphoxide, $\text{NH} \begin{array}{c} \text{C}_6\text{H}_3\text{NO}_2 \\ \text{C}_6\text{H}_3\text{NO}_2 \end{array} \text{SO}$, in 100 c.c. of decinormal sodium hydrate solution) are then added and the solution boiled for one or two minutes, then diluted to 100 c.c. with water, and filtered by suction. If tin is present the solution turns violet during filtration, the full depth of colour being attained by the addition of a drop of ferric chloride solution. The process, after obtaining the sulphide, only takes a few minutes. The amount of tin present is found by comparison with a standard solution of tin chloride (containing 14.28 mgm. of tin per 100 grms. or the equivalent of 1 grain per lb.). The colour, due to the formation of Lauth's violet, is not strictly proportional to the amount of tin present. One-tenth of a grain of tin per lb. gives an appreciable colour, while with over one grain it is advisable to take less than 10 grains of foodstuff or estimate the tin gravimetrically.

The table on page 374 gives an abstract of the results of Dr. Schryver's examination of various canned foods.

Commenting upon the results, Dr. Buchanan states that meat extracts and essences, owing to their natural acidity, take up tin to a greater extent than other meat products. From the same cause canned fruits and vegetables are also specially liable to take up tin, the metal may penetrate into the substance of the solid food, which may come to contain relatively larger proportions of tin than the liquid. If solder gains access to the interior of the can, a very conspicuous solution of tin may take place. The results obtained by Dr. Schryver, from experiments on himself and animals, are briefly as follows :—

That there is no evidence of a cumulative action of tin until the daily dose exceeds 2 grains.

Foodstuffs.	Origin.	Grains of tin per lb.
Bacon, sliced	U.S.A.	0.61
Beef essence	England (3 makers)	1.58 to 1.92
Beef extract	S. America (2 makers)	0.40 „ 5.33
Curried rabbit	Australia	0.19
Fruits	London importer	0.33 „ 1.03
Jams	{ England	1.42 „ 2.81
	{ U.S.A. (tin pierced)	5.13
Lobsters	U.S.A.	2.39
Plum-pudding	England	trace
Pork-pie	England	2.92
Roast fowl	England	0.58 „ 1.44
Salmon	British Columbia	0.4 „ 0.6
Tomato soup	U.S.A.	3.5
Vegetables	Australia	1.51 „ 2.19

That there is relatively small amount of absorption of tin from the alimentary tract.

The experiments support in the main Lehmann's conclusions, that there is not much probability of serious risk of chronic poisoning from a diet consisting largely of canned foods and continued over considerable periods of time.

Dr. Buchanan states, however, that the presence of tin in a sample can in quantities approaching 2 grains to the pound may be taken to signify that the food has become potentially deleterious to health, and calls for the examination of further samples.

F. Wirthle ("Chem. Zeit." xxiv. 263), reports on the examination of samples of preserved meats of various ages up to five years. The metal of the tins contained only 0.21 per cent of lead, and there was no soldered joint. The amount of tin present was found to increase with the time of preservation, and the meat to contain three times as much tin as the juice. The interior of the tins was found to be corroded almost solely where there was an accumulation of fat. They were not acted on where they came in contact with gelatin. In five-year-old tins a white crust was formed which consisted of basic tin chloride, due to the action of the sodium chloride present on the tin. In four-year-old tins a black layer of sulphide was present. The tin was determined in the meat and juice, by the following modification of Orfila's method: About 120 grms of meat (or juices, separated from the meat) were placed in a large porcelain dish of nearly 1 litre capacity, moistened with 5 c.c. concentrated sulphuric acid, and heated carefully on a sheet of asbestos. It was frequently stirred, and from time to time small quantities of sulphuric acid were added, altogether about 15 c.c. to 20 c.c.; the mass was occasionally removed from the sides of the dish, to which it adhered, by means of a porcelain spatula. After four or five hours, a porous carbonaceous mass was thus obtained, which was pulverized and incinerated in a porcelain crucible. The particles adhering to the porcelain dish were transferred to the crucibles with the assistance of powdered anhydrous carbonate of soda;

a further proportion of carbonate of soda was added, together with a sufficient quantity of nitrate of soda, the whole thoroughly mixed, and heated to gentle fusion. After cooling the melted mass was taken up with water, and when the cloudy solution had become clear (which generally takes place after about twelve hours), the precipitate was collected on a filter, well washed, dried, and incinerated. The ash was treated with a sufficient quantity of potassium cyanide, and the mixture heated to dull redness, the crucible being closed. The melted mass was taken up with warm water, and the metallic tin and the iron collected on a filter, washed, and dissolved in a little warm hydrochloric acid. In the solution, which should not be very acid, the tin was precipitated with sulphuretted hydrogen, the precipitated sulphide was washed with water, saturated with sulphuretted hydrogen, containing a small quantity of nitrate of ammonium, and then dried, incinerated, and calcined until the weight was constant. The weighed stannic oxide was reduced once more by means of potassium cyanide; the tin thus obtained was dissolved in hydrochloric acid, precipitated in the form of sulphide, and weighed as stannic oxide. The minimum amount of tin found in the meat was 0.0029 per cent, and in the juice 0.0011 per cent. The maximum was 0.016 per cent in meat, and 0.0036 in the juice.

Allen detects heavy metals according to the following scheme:—

The substance to be examined is heated on a water bath, and finally at a rather higher temperature with sufficient strong sulphuric acid to well moisten the whole of the substance, with which it is incorporated; 1 c.c. of HNO_3 is then added, and the heating continued until red fumes are given off. Ignited magnesia (0.5 grm. for each grm. of acid used) is now well mixed with the substance, and the whole ignited at a dull red heat. After cooling, the ash is moistened with nitric acid and reignited. This treatment is repeated until the ash is white or grey. Ten drops of H_2SO_4 are added, the whole heated until fumes are evolved, cooled, boiled with water, diluted to about 100 c.c. and without filtering, treated with H_2S to saturation. The solution is now filtered and the following scheme of analysis followed:—

Aqueous solution may contain zinc and iron. Add Br. water to destroy H_2S . Add excess of NH_3 , boil, and filter again.		Ppt. may contain Pb, Sn, or Cu. Fuse in porcelain with 2 grs. of sodium-potassium carbonate and 1 gr. of sulphur. Cool, boil with water and filter.	
Ppt. may contain iron.	Filtrate, if blue, contains nickel. Divide into two parts.		Residue. Boil with strong HCl , add Br. water. Filter. Add NH_3 —a blue colour indicates copper. Acidulate with acetic acid and divide into two parts. To one add potassium bichromate, a yellow ppt. indicates Pb. To the other add potassium ferrocyanide, a brown ppt. indicates copper.
	1. Heat to boiling and add potassium ferrocyanide white ppt. = zinc.	2. If zinc be found in 1, determine it by adding acetic acid and precipitate with H_2S . Nickel if present will be included.	
			Filtrate. Add excess of acetic acid, a yellow ppt. indicates Sn.

Exceedingly minute amounts of copper may be detected by inserting a bright steel needle into a slightly acidulated concentrated extract of the ash, removing it after some hours, cautiously rinsing with water and then immersing it in ammonia with free contact of air. Copper will be detected by acidulating the ammonia solution with acetic acid and adding potassium ferrocyanide when a brown colour or precipitate will be formed.

Preservatives in Tinned (or glass-contained) Meats.—The most important publication dealing with this matter that has recently appeared is the report of Dr. A. W. J. MacFadden to the Local Government Board of 26 May, 1908, with an analytical addendum by P. A. Richards. The following are the most important portions of this report:—

During the summer and autumn of 1906 a large number of public analysts throughout the country received samples of canned and glass-packed meats, and in response to a request by Dr. Buchanan furnished special reports on the result of their examination of these foods. From these reports it was noticed that the percentage of samples in which preservatives were found was much higher than might have been expected having regard to the fact that the cans or glasses in which these articles were packed had been hermetically sealed and, presumably, sterilized by heat in the usual way. Out of a total of 1733 hermetically sealed tins or glasses dealt with in the above-mentioned returns, no fewer than 333, or over 19 per cent, were reported to contain chemical preservatives other than salt and saltpetre. Of these preservatives, 243 were boron compounds, forming 14 per cent of the whole, and in the remaining 90 samples the presence of sulphite preservatives was reported.

In the case of meat foods of this kind it is generally understood that, with certain possible exceptions, there should be no need for the addition of chemical antiseptics at the time of preparation of the meat for canning, and that the meat which is canned should ordinarily be fresh meat, or cured meat—not meat which has been subjected to treatment involving the introduction of preservatives such as boric acid or sulphites. It is to be expected that meat foods of this kind which have been submitted to a process of sterilization in hermetically sealed containers should be sufficiently protected by these means from processes of decomposition so long as they remain unopened; chemical preservatives are not required to further this object.

In these circumstances the use of preservative materials of the kind referred to appeared liable to objection, apart from any risk to health arising from consumption of the preservatives themselves, in that their presence pointed to the probability that by this means it had been sought to overcome undesirable conditions either in the meat itself or in the processes of its manufacture.

The following general conclusions are drawn:—

(1) PRESERVATIVES IN IMPORTED CANNED MEAT FOODS.

The finding of preservatives in a considerable proportion of American and other imported canned meats, examined by public analysts in

this country, is a matter of importance from a public health aspect. The question is not merely one of the ill-effects likely to be produced in persons who, in this way, consume what may possibly be large doses of these substances or who, by taking these foods, add unnecessarily to the total quantity of antiseptic substances in their diet. The chief objection which may be raised to preservatives occurring in meat foods which have been subjected to the process of sterilization by heat in hermetically sealed vessels, is that the presence of preservatives must be regarded as indicating that conditions as to care and cleanliness which are essential in the preparation of wholesome food materials may not have been observed.

Dr. Eugene H. Porter, Commissioner of Health of the State of New York, has referred to this matter in the following terms :—

“The use of any preservative in a food to be enclosed in a can which can be satisfactorily sterilized by the use of heat and sealed hermetically, indicates that the materials to be placed in the can were in such a state, or were kept under such conditions, as to lead the canner to believe that they required the use of a preservative for the prevention of decomposition until they could be safely canned.

“The finding of a boron preservative in a sample of “potted ham,” in which were found numbers of the *Trichina spiralis*, clearly indicates that in the minds of those who prepared this meat, it required a preservative in order to prevent its decomposition before it could be sterilized in the can.

“It is not easy or always possible to ascertain from an examination of a sample of canned meat containing a preservative whether decomposition had set in at the time the preservative was added or not, but the possibility or the probability of the development of such undesirable changes must have been present or the preservative would not be added.”

The views expressed above undoubtedly offer the correct explanation of the circumstances which lead to the presence of boron compounds in foods of this kind in the great majority of cases, and it is difficult to accept the explanations in this matter which are commonly given by representatives of canning firms.

Similar considerations may be said to apply to sulphite preservatives. These seem to have been reported more frequently in American canned meats than in those of British manufacture, and though there are chemical and analytical conditions which impair the value of quantitative analytical results obtained in regard to them, the fact that sulphites are frequently found in canned meats cannot seriously be questioned, and their presence can only suggest the same interpretations as that just given.

Extension of Protection Afforded by Foreign and Colonial Laws.—The United States Meat Inspection Act of 1906, which was the outcome of inquiries made into this, together with other aspects of the American meat-packing trade, prohibits the use of artificial preservatives in meat and meat food products, and it may be hoped that the very comprehensive and stringent provisions contained in that measure are already having, or will have, the effect of abolishing those

practices which lead to the use of chemical preservatives in canned meats.

Legislation as to supervision of the preparation of meat foods in various States of Australia, in New Zealand, and more recently in Canada may also be alluded to as probably affording a substantial check on the use of preservatives in canned meat foods which are subject to official supervision in those Dominions.

It must, however, be remembered that the extent to which foreign and colonial laws and regulations regarding canned foods are likely to be and to remain operative in practice in the case of the exports to the United Kingdom, depends very largely on the efficiency of precautionary measures taken in this country. At a time when systems of official inspection, regulation and certification in connexion with the manufacture and preparation of meat foods are being rapidly developed in foreign countries and British dominions, there would appear to be special advantage in specifying British requirements in such a way that foreign and colonial manufacturers and officials are in no doubt as to what the public health authorities in Great Britain expect them to avoid or to do in regard to the use of preservatives in canned meats exported to this country, and in arranging some systematic method of analytical control for the purpose.

(2) PRESERVATIVES IN MEAT FOODS CANNED OR PACKED IN GLASS IN THE UNITED KINGDOM.

Preservatives in the Raw Materials.—Boric acid in large amount has been found in canned meats of British manufacture, as the result of employing meats imported from abroad in this preservative. Convictions have been obtained by local authorities under the Sale of Food and Drugs Act in such cases. But the number of instances coming to the notice of these authorities in which amounts of boric acid are sufficiently large to ensure successful prosecution is necessarily small and forms but a slight check on the practice of employing such meats for canning purposes.

The second and larger class of meats which come under this head are bacon and hams. These are meats which in normal circumstances are cured by means of salt and saltpetre, and occasionally sugar. The process of curing renders the meat more or less immune to the influences of putrefactive organisms according to the length of time during which the meat has been kept in "pickle". It was the custom formerly to continue the curing process for a sufficient length of time to ensure that when removed from the pickle and properly dried, hams and bacon would remain sound for long periods. They were, however, liable to be affected in various ways where conditions as to curing or storage were faulty. Thus putrefactive processes like "taint," and contaminations such as flyblows were accidents which required constant guarding against by bacon curers.

When borax came into use as a preservative it was found that these conditions were prevented by sprinkling the surfaces of the meat with powdered borax. Later this substance came to be used as a con-

stituent of the pickle, and it was found that by employing the preservative in these ways the curing process could be shortened without to a corresponding extent endangering the keeping qualities of the meat. The lessened salty flavour of hams and bacon which followed the introduction of these methods appears to have met with favour among consumers generally, and it has since been the aim of most bacon and ham curers to produce materials as mildly cured as possible. In these circumstances the use of boric acid has come to be regarded by many bacon manufacturers as a necessity, more especially where their products have to be kept for long periods or are intended for distant markets. On the other hand, some well-known British manufacturers declare that no such necessity exists, and that they are able to prepare, store and export their products to all parts of the world without having resort to boron preservatives, or other antiseptics, at any stage.

Before the use of borax was introduced for the purpose mentioned above, hams and bacon were sent from the United States to this country packed in salt. This method of transport amounted practically to a continuation of the curing process for a number of weeks longer than was the custom in the case of home-cured materials and, owing to their consequent increased saltiness, American hams and bacon suffered greatly by comparison with British produce in the home market. The use of borax in the manner above mentioned has enabled American and Canadian curers to send their produce to Great Britain in a condition which, as regards "mildness" of flavour, complies with the taste of consumers in this country.

Borax-packed Hams and Bacons as a Source of Preservative in Canned Meats.—The amount of borax acid found in bacon and hams imported under the above conditions will depend on whether the preservative has been employed only as a packing material, or has in addition been used in the curing of the meat. Examination of samples of hams said to have been only packed in borax shows that amounts varying from 2·6 grains to 13·5 grains per pound of the minced and mixed ham may result from mere contact with the preservative in the packing cases. Larger quantities than this, up to 24 grains per lb. of boric acid, were mentioned to the Departmental Committee on Preservatives and Colouring Matters as having been found in hams, and the Committee refer to this as an indication that boric acid had been used in the curing of these products.

Since the passing of the United States Meat Inspection Act the use of borax in hams and bacon sent to this country is said to have been confined to external application as a packing material. Generally it may be said that this use of borax is free from the objection that the preservative has been used to mask the effect of objectionable conditions of preparation, etc. It may be sufficient in this connexion to observe that if the use of boron preservative in food were to be restricted with the object primarily of limiting the number of foods which at present may contain this preservative, and were to be confined to such foods as in present circumstances appear to demand borax as an essential condition of trade, a good case would be made

for permitting the continuance of borax packing in the case of American and Canadian hams and bacon, provided the amount of preservative absorbed by the meat was not excessive. On the other hand no trade necessity can be alleged for using borax in the curing process itself, a view which is borne out by the fact that for some time past American manufacturers have apparently been able to dispense with this use of the preservative.

The amount of preservative which may be found in certain varieties of canned meats, due to their having been manufactured from hams and bacon in which boric acid had been employed solely as a packing material, would in most cases be small and of little importance as a public health consideration. But it would be necessary to take account of such small quantities of preservative in any scheme which might be arranged for controlling the use of preservatives in canned meats. If potted meats made from imported hams were exempted from any restriction as regards preservatives that might be applied to canned meats in general, such exemption would be certain to afford a convenient plea for manufacturers who desired to continue the improper use of boric acid in potted meats, and the difficulty of administration would be greatly increased in such circumstances. It should be remembered in this connexion that hams and bacon which are free from preservative can be obtained in British markets, and that some well-known manufacturers, in order to ensure the freedom of their products from boric acid, have already taken the precaution of using only those American hams and bacon which are sent to this country in salt packing and guaranteed to be free from chemical antiseptics. Apparently an unlimited supply of these salt-packed hams could be had if demanded, and for a price not greater than that charged for borax-packed materials. Danish bacon free from borax is also available, and it could no doubt be arranged by potted-meat makers who desired it, to obtain bacon and hams of British origin which did not contain any preservative of this kind.

Addition of Preservatives by the Canner.—As already stated the presence of boric acid in canned goods of British manufacture may to a certain extent be attributed to sources referred to in the foregoing. In a great number of instances, however, the presence of preservatives in these materials cannot, for various reasons, be explained in this way. Such is the case with, for example, many of the "potted meats" which have been shown by analysis to contain exceptionally large quantities of boron preservative. Notwithstanding the fact that these materials are in most cases mixtures of two or more meats and often contain in addition a large percentage of farinaceous matter, the amounts of boric acid found in them have frequently been much greater than is ever likely to be found in raw material of the kind above-mentioned from which they might have been made. The only possible explanation of the presence of preservatives in such circumstances is that they have been deliberately added to the material in process of manufacture.

It has been indicated in section 3 of the report that the use of meat which has become, or is on the point of becoming, tainted may

lead to the use of chemical antiseptics (as by immersing in fluids, spraying or powdering) for the purpose of preserving it. It has also been shown that even where sound materials are used to start with, faulty, dilatory and uncleanly methods of manufacture may so far affect the condition of the meat previous to packing that the use of antiseptics has to be resorted to.

It will be gathered from descriptions given in preceding pages that the various steps in the manufacturing process of canned and glass-packed goods are operations which require considerable skill and care. In handling the perishable material dealt with, great cleanliness is needed and every convenience for securing this and for enabling the various steps to be carried out with despatch, is essential if the material is to reach the consumer in a wholesome condition. In the majority of the factories visited, care seemed to be taken to ensure that these essential requirements were observed. Reference has, however, been made to instances in which one or other important point was not attended to. A few of the factories were, indeed, quite unfitted for the use to which they were put. Places hidden away among the dilapidated buildings of dingy streets or beneath remote railway arches—sometimes even within the same curtilage as premises in which offensive trades are conducted—cannot be considered fit for the preparation of food material intended for human consumption. The structural defects of such places are sufficient in themselves to render the cleanly preparation of canned meats in them impossible, but when, in addition, as is frequently the case, the appliances used at these factories, and the workpeople who use them, are of no higher standard of cleanliness than the buildings themselves, it is not difficult to understand the need which arises for means which will counteract in some way influences so adverse to the preservation of the meat used.

I found many grades of greater or less efficiency between such entirely undesirable food factories and others which could be pronounced without hesitation to be satisfactory in all essential respects. But neglect of some obvious precaution, such as the necessity for carrying out certain stages in the manufacture speedily, and under suitable conditions of temperature, etc., may render unavailing the care taken in other steps of the process, and may lead to the addition of preservatives at this stage.

There is a tendency on the part of some manufacturers to leave the technical details of their business too much in the hands of the workmen who carry them out. Many of these workmen look upon the details of the processes which they are conducting as technical secrets of their own, and resent any interference with the preconceived notions which they may have regarding them. A manufacturer who has not a well-grounded knowledge on these matters, or who, while possessing this, fails to insist on his ideas being carried out, is at the mercy of his foremen. The latter are in some cases content to proceed with the methods in which they were brought up, and are naturally inclined to continue practices which protect them from the consequences of miscalculations or mistakes. The use of antiseptics from their point of view, possesses this advantage, and it is not to be

wondered at that when given a free hand, they should avail themselves of it.

Greater Use of Preservatives in Glass-packed Meats.—The use of chemical antiseptics is especially undesirable in the case of meat foods packed in glass, which have lately come into much favour with the public. Many complaints were made during my inquiry as to the difficulties which had to be overcome in connexion with the manufacture of meats so packed. The chief of these was the difficulty experienced in the sterilizing process, of steering a middle course between the risk of breakage on one hand, and that of insufficient sterilization on the other. This is doubtless an operation requiring care and experience. Most manufacturers, however, have been able to carry it out with success, so far as the conferring of keeping qualities on their meat is concerned, and some of them have even stated that they experience no difficulty whatever in the matter. The question seems to be entirely one of efficient technique, and to avoid the danger by adding sufficient antiseptic to preserve the packed material against the consequences of faulty sterilization, amounts to a pretence and cannot be justified. A still more unjustifiable practice is that which has been referred to of adding boric acid to potted meats hermetically sealed in glass, in order to avoid sterilizing them at all. To sell meats prepared in this way in hermetically sealed containers, which purport to be preserved by means of heat, or are of a pattern which the purchaser generally associates with goods preserved in this way, is a form of deception which may be prejudicial to the health of persons consuming them.

The alleged inefficiency of certain forms of metal caps for producing a hermetic seal with the glass containers for which they are designed is, I am convinced, unreal. Complaints as to this were made only by those who, either from fear of breakage, or in order to preserve the homogeneous appearance of their potted meats, sterilized these products imperfectly or omitted the process altogether. Many of the better manufacturers had no difficulty with these covers, and were able to rely with considerable certainty on their process of sterilization alone to preserve the meats sealed by means of them.

It is undoubtedly more difficult to produce meats properly preserved by means of heat in glass containers than in cans. The high temperature to which canned materials may be subjected for long periods without risk renders the sterilization of such meats a much more certain process than is the case with glass-packed goods. None of the manufacturers whom I saw had experienced any real difficulty in preserving their canned products by means of heat, and all denied using preservatives, or the necessity for using them to supplement this process in canned goods. To this extent canned meats in general might be said to possess advantage, from the point of view of wholesomeness, over meats which are packed in glass. On the other hand, the difficulties met with in sterilizing glass-packed meats necessitate the observance of greater cleanliness and care in their preparation (*so long as preservatives are not added*) if satisfactory results are to be obtained. Added to this is the fact that their contents are more or less

capable of inspection by the purchaser, and for this reason very unlikely to contain any of the grosser contaminations which have been reported in some American canned foods, for example.

The freedom of glass-packed meats from liability to metallic contamination is another point which may be noted in their favour, though the practice among some British manufacturers of re-packing in glass imported meats which have just been turned out of their cans for the purpose, is calculated to shake the confidence of those who rely for protection in this respect on buying only meat preserved in glass containers.

(3) ADMINISTRATIVE CONSIDERATIONS IN REGARD TO PRESERVATIVES IN CANNED AND GLASS-PACKED MEATS (*IMPORTED OR HOME-PREPARED*).

On review of the above, it seems desirable that steps should be taken to secure that specified chemical preservatives should not be used in the preparation of canned meats intended for consumption in this country. In any schedule of prohibited preservatives, boron compounds, sulphites and preparations of sulphurous acid, benzoic acid and formalin should, I think, be included.

The action which for this purpose may be recommended for the Board's considerations is such as could apparently be made available by the issue of suitable regulations under the Public Health (Regulations as to Food) Act, 1907, made applicable (1) to imported canned foods at the ports of entry; (2) to manufacturers in this country of meat foods which are packed in cans or glass. Should regulations be prepared with this object (either as a separate series or as part of a larger series dealing with preservatives in a variety of food-stuffs), it would seem desirable to make due allowance for trade requirements in the matter of existing contracts, stocks on hand, and so forth.

(4) ADDITIONAL OBSERVATIONS.

In concluding this section attention may be directed to certain matters which have incidentally come into prominence as a result of my inquiries:—

(a) *Proprietary Antiseptic Preparations*.—It seems desirable that manufacturers of meat foods should refuse to purchase any preparations offered to them for the purpose of treating meat in storage or of adding to meat preparations or brines unless they are fully assured as to the composition of any preparations so offered.

(b) *Labelling*.—So far as my inquiry has been concerned with the question of labelling and marking of canned foods, it has brought into prominence the fact that the custom which at present prevails in this matter in many cases does not permit the origin of particular tins of canned meats to be traced further than to the middleman or importer. In some cases (including to my knowledge at least one in which the wholesomeness of the foods was open to very serious question) neither the can nor the label bears any name or other mark

by which it can be referred to retailer, middleman, manufacturer, or anyone at all. Reform in the trade custom in this respect seems on many grounds desirable.

(c) *Admixture of Starch with Potted Meats.*—Starch, usually in the form of rice flour, is sometimes present in such amounts in some kinds of potted meats that these may be thought to constitute a fraud on the consumer notwithstanding their cheap price. The matter seems worth the attention of better-class manufacturers with a view to arriving at a reasonable maximum limit of permissible starchy matter in specified canned foods of this class.

(d) *Preservatives in Sausages, etc.*—Some of the objections above set out to the use of chemical antiseptics in canned meats apply also to such articles as sausages, pork pies, mincemeats, brawns, and the like, which are not preserved by canning or cold storage, but in the ordinary course of trade may be kept for several days on the shop counter and similar places. If on the ground of public convenience and trade requirements the use of chemical antiseptics is permitted in these articles, it appears very desirable that their employment should be restricted within narrow limits. Quantities of boric acid are not seldom reported by public analysts in some of these goods which cannot be otherwise than prejudicial in themselves, besides being wholly unnecessary. If boron preparations are used for this purpose, a limit of $\frac{1}{4}$ per cent of boric acid would probably be ample to meet legitimate trade requirements, and even in this case it appears desirable to consider whether notification of the presence of the preservatives should not be given to the purchaser. The practice of using solutions of sulphurous acid or sulphites as a spray or wash, or for mixing with the meat, appears to be open to many abuses and to be generally undesirable.

(e) *Places where Food is Prepared.*—The Board have recently received representations from several sources to the effect that stricter sanitary supervision and control should be exercised by local authorities in this country over premises on which meat foods are manufactured and over the processes of preparation employed on such premises. It has likewise been urged that all practicable steps should be taken to require evidence from official sources as to the inspection or supervision in foreign or colonial establishments whence meat foods are imported into the United Kingdom. The inquiries above recorded have brought out many circumstances which appear to support these views.

The United States Department of Agriculture in a bulletin published in 1907 (No. 76—Foods and Drugs Inspection Section) decided that no drug, chemical, or harmful or deleterious dye or preservative may be added to foods, or used in preparing them for the market, except common salt, sugar, wood-smoke, potable distilled liquors, vinegar, condiments, and, until further investigation, saltpetre. Sulphur dioxide is permitted within limits for wines and food-products provided the amount does not exceed 350 mgrms. per litre in wines, or per kilogram of food-products, but not more than 70 mgrms. should be in the free state. Sodium benzoate not ex-

ceeding 1 per mille or an equivalent amount of benzoic acid may be used as a food preservative, and in this case, as well as sulphur dioxide, the fact must be stated on the labels. The effects of coal-tar dyes in foodstuffs are being investigated, and until the investigation is complete they propose to permit the use of the following as noted in Professor A. G. Green's edition of the "Schultz-Julius Systematic Survey of the Organic Colouring-matters" published in 1904:—

Red Shades: 107. Amaranth. 56. Ponceau 3 R. 517. Erythrosin.

Orange Shade: 85. Orange I.

Yellow Shade: 4. Naphthol yellow S.

Green Shade: 435. Light Green S. F. yellowish.

Blue Shade: 692. Indigo disulphoacid.

Each of these colours shall be free from any colouring matter other than the one specified, and shall not contain any contamination due to imperfect or incomplete manufacture.

The various preservatives present in preserved foods may be searched for by the methods described under milk (p. 160), wine (p. 332), and meat extract (p. 413).

The use of benzoic acid has been common of recent years as a preservative, especially in certain American preparations. The preparation—if solid or semi-solid—should be well extracted by macerating it with dilute alkali, straining through fine muslin and then acidifying and extracting with an immiscible solvent as in the case of salicylic acid. The ether or chloroform containing the free acid may be, in turn, extracted with dilute ammonia, and the liquid evaporated nearly to dryness, and the concentrated liquid tested. To a few drops, a drop of neutral ferric chloride is added, when a characteristic flesh-coloured precipitate is thrown down. In case the food contains an artificial colour, which might mark the reaction, Mohler's test may be applied. The ether extract is dried and the residue heated with 2 c.c. of strong sulphuric acid, which converts it into sulphobenzoic acid. A few crystals of KNO_3 are then added, which causes the formation of meta-dinitro-benzoic acid. When cold, the acid is diluted with water, and ammonia added in excess, and then a few drops of colourless ammonium sulphide solution. A red colour is at once developed owing to the reduction of the acid to meta-diamido-benzoic acid, whose ammonium salt is red.

A useful confirmation (in the absence of salicylic acid and saccharin) is obtained by dissolving about 0.1 gm. of the ether residue suspected, in 5 to 6 c.c. of H_2SO_4 . A small quantity of barium peroxide is then added, the tube being immersed in cold water, as fragments are successively added, in all about 0.75 gm. After standing for half an hour, the liquid is diluted with water and extracted with ether, the residue being tested for salicylic acid, into which the benzoic acid has been converted, in the usual manner.

Benzoic acid may be determined by the method of La Wall and Bradshaw ("Amer. Jour. Pharm." **80**, 1908, 171). Twenty grms. of the material are well mixed with 2 grms. of sodium chloride, 5 c.c. of HCl . and 25 c.c. of brine. The whole is well mixed and shaken for

ten minutes. Transfer to a moistened filter and after the liquid is drained, the residue should be treated with three more quantities of 25 c.c. of brine, and drained into the filter each time, being washed with more brine till the filtrate measures 100 c.c. Shake out the filtrate with three portions of chloroform (25, 15 and 10 c.c.). Allow the chloroform to evaporate at ordinary temperature, and dry to constant weight in a desiccator. The result should be confirmed by titrating the residue dissolved in a little alcohol, with one-twentieth normal KOH.

Sausages.—Sausages, although made to a considerable extent in this country, are essentially a German delicacy, and the German sausages imported into this country are many in number.

In this country sausages are principally made from pork and from beef with certain spices and condiments, usually some colouring matter, and frequently bread or other starchy material. The so-called "German" sausages made in this country resemble no true German sausage that the author has ever examined.

The following are the principal types of sausage manufactured in Germany (most of which find their way to this country), after the classification of König and by Merges.

Rothwurst (or *Buntwurst*) resembles the English "black pudding". It is made from pork, bacon, often with the addition of heart or kidney, various spices and frequently amylaceous material.

Mettwurst is made from pork, with a large addition of lard, frequently beef and horse-flesh. It is frequently coloured with coal-tar dyes.

Cervelatwurst.—This is generally made from the brains of pigs and horses, with the addition of pork and lard, and usually a little colouring matter.

Leberwurst.—This is made from the livers of pigs and calves, with the addition of pork and lard. Frequently the liver and lungs enter into the composition of this sausage, as well as some starchy matter.

Magenwurst is made from the stomach, skin and other parts of the pig with blood and unsalted bacon.

Bratwurst is made from raw pork, and bacon with lemon and cumin as flavourings.

Erbswurst is made from suet, bacon, pea-flour, onions and various other seasonings.

Frankfort sausages are small sausages made of raw pork and seasonings.

The following are typical analysis of several types of German sausages purchased and examined by the author:—

	Water.	Fat.	Carbohydrates.	Nitrogen.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent
Leberwurst	43.5	24.5	10.6	2.3	5.4
Mettwurst	27.4	36.9	7.3	3.26	6.0
Cervelatwurst	33.8	41.0	2.8	2.74	4.95
Frankfort sausages	40.8	31.9	6.5	2.45	4.16

König has published a number of analyses of these and other sausages, but they all have similar compositions to the above.

Allen gives the following as the composition of average quality English-made sausages:—

	Water.	Fat.	Proteids.	Gristle, etc.	Starch.	Ash.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Pork	54.99	21.04	12.88	0.67	1.05	3.52
Mutton	55.58	30.57	1.89	3.11	3.90	2.50
"German"	49.54	17.87	16.38	1.13	15.00	4.47
Poloney	45.57	32.66	17.26	0.54	2.30	2.80

As a rule the determinations required in the examination of sausages are those of preservatives and artificial colouring matters, with a further examination for the presence of parasites, and, occasionally, for the presence of horse-flesh. A fuller investigation is sometimes required, however, when the following methods will be found sufficient:—

Moisture and Ash.—This is determined by drying 10 grains at 105° to 110° to constant weight. The ash is determined by drying and incinerating 2 to 3 grms.

Fat.—The fat may be determined by extraction of the above dried residue (preferably rubbed down with sand) with ether in a Soxhlet tube, if an examination of the fat be necessary, a larger quantity must be extracted. The following are the typical characters of the commoner fats met with in sausages:—

	Ox.	Sheep.	Pig.	Horse.
	Per cent	Per cent	Per cent	Per cent
Sp. gravity $\frac{100^\circ}{15}$	0.860	0.860	0.860	0.861
Saponification value	192 to 198	192 to 196	194 to 196	195 to 198
Iodine value	36 „ 44	34 „ 44	50 „ 68	75 „ 85
Reichert value	0.25	0.3	0.4 „ 0.6	0.8 „ 1.1
Refractive index at 60°	1.4510	1.4500	1.4540	—
Butyro-refractometer No. at 40°	48 to 49	48	50 to 51	53 to 54

Starchy Matter.—A small portion of the sausage may be teased on a microscopic slide and a drop of iodine solution added. The presence of starch is easily seen by observing the black stained starch granules under the microscope.

Mahrhofer ("Analyst," xxii. 2) determines starch in the following manner: From 60 grms. to 80 grms. of the sample are heated on the water bath with alcoholic KOH (10 per cent). Nearly everything is dissolved. The solution is diluted with warm alcohol in order

to prevent gelatinization, and then filtered. The insoluble residue contains the starch, if any be present, and is washed with alcohol until free from alkali, and then treated with aqueous potash, which dissolves the starch, and made up to a definite volume. An aliquot portion is precipitated with alcohol, when the starch can be collected on a dry tared filter, washed with alcohol and ether, dried and weighed.

Medicus and Schwab ("Berichte," xii. 1285) recommend the digestion of a weighed quantity of the sample for ten hours with a definite volume of an infusion of malt, at 30° to 40° . The mixture is then allowed to stand for eighteen hours at ordinary temperature, and then filtered, the insoluble matter washed, the filtrate boiled, and the precipitated albumen filtered off. The filtrate is then boiled with HCl to convert the dextrin and maltose into dextrose, which is determined in the usual manner by Fehling's solution. The amount in the volume of malt infusion used, is determined, and deducted from the result. Ten parts of dextrose may be taken as representing 9 parts of dry starch originally present. It must be remembered that a very small amount of starch will usually be present on account of the pepper added to the sausage meat.

Determination of Total Nitrogen.—Two grms. of the sample are submitted to the Gunning or Kjeldahl method of treatment. In the case of meat the customary method of taking $N \times 6.25$ as representing the total protein or nitrogenous substances, cannot always be relied upon, on account of the varying amount of nitrogenous matter contained in various compounds present, though a fairly close approximation of the nitrogenous substance present can be calculated by making use of this factor, as proteins are by far the largest group contained.

Separation and Examination of Nitrogenous Bodies.—It entirely depends on the nature of the sample in hand how far an analyst should subdivide the various nitrogenous bodies present in meat. The above-mentioned simple determination of total nitrogen is frequently sufficient. As a rule there is no necessity to do more than divide the nitrogenous bodies into several main groups according to their solubility in water or other solvents, and their attitude towards certain reagents. Nitrogen can be determined separately in each of these classes, and the corresponding nitrogen substance or class of substances can be obtained by the appropriate factor.

To more completely separate the various classes of nitrogenous bodies found in meat, agitate a portion of the fat-free sample with cold water to remove the soluble proteins (soluble globulins, proteoses and peptones) and meat bases, leaving behind the insoluble globulins, the sarcolemma, the albuminoids of the connective tissue and the collagen. Then treat with boiling water, thus removing collagen in the form of soluble gelatin. The soluble proteins, including the peptones and gelatin, can be precipitated from the meat bases by adding zinc sulphate, sodium chloride, and tannic acid to the combined aqueous extract.

Determination of Nitrogenous Substances Insoluble in Water.—Thoroughly wash the sample with cold water, transfer the filter and

insoluble material to a flask, then determine the nitrogen by the Gunning or Kjeldahl method. Multiply the insoluble nitrogen thus obtained by 6.25 to obtain insoluble proteins. The insoluble nitrogen can also obviously be obtained by deducting the soluble from the total nitrogen. Dilute the cold water extract to definite volume, determine the nitrogen in an aliquot portion, and calculate to percentage of soluble nitrogen in the weight of total extract. Having obtained the percentage, deduct it from the percentage of total nitrogen, and the result is the percentage of insoluble nitrogen.

Trowbridge and Grindley take a sample previously ground in a meat chopper, and immerse it for one hour in ice water, in the proportion of 1000 grms. of meat to 1500 c.c. of water. This solution is then filtered through a cheese cloth, at the same time assisting the process by squeezing the cloth with the hand. The residue thus obtained is divided into smaller portions, transferred to beakers washed in series, fresh water being used with No. 1 only, filtering through cheese cloth from one beaker to another until the last filtrate is colourless, neutral to phenol-phthalein, and gives no reaction for proteins by the biuret test. The mixed filtrates and washings easily filter through paper giving a clear red filtrate, in which soluble nitrogen can be determined.

Pennington employs the following process with the meat of chickens: Place a portion of the finely divided red or white meat, 60 grms. in weight, into a tall slender bottle of 500 c.c. capacity, made to fit a centrifuge which can hold 1 litre of material; add 300 c.c. of water, and shake the flask gently for fifteen minutes. This movement is only sufficient to keep the particles of meat in motion and the composition of the extract homogeneous. An emulsion is formed when the shaking is violent and when the tissue is very finely ground. Having shaken for the specified length of time, rotate the flask in a centrifuge for twenty minutes, thus causing the heavier particles to settle in a compact mass, and allowing the decantation of the liquid floating on the top, which should be then filtered through paper. Repeat the extraction as outlined, with portions of 300 c.c. of water until the filtrate is practically free from protein as indicated by the biuret reaction. A volume of 1500 to 2500 c.c. is generally necessary to obtain this result. Add thymol to both the flesh and the extract to prevent bacterial decomposition, and keep cold, using ice if necessary to keep the meat immune from the naturally occurring enzymes.

The extraction of the white meat is a much simpler process than the extraction of the dark meat. The latter does not settle so compactly after rotating in the centrifuge, it is slower in filtering and continues to show a distinct biuret reaction for a long time after the white meat is freed from water-soluble proteins. Certain fowls in fact, especially those kept in cold storage for a considerable time, never show a dark meat completely free from water-soluble nitrogen. In these cases, the question of the error owing to long handling and enzyme action, causing an increase in the actual quantity, has to be taken into consideration. It has been noticed after experiment that

when there has been a long extraction of such tissue, a point is reached when a very faint biuret reaction appears indefinitely and does not seem to diminish. These extractions are continued for about twenty-six hours, as it is probable that a greater error would arise in the gain of what has been originally insoluble material, than the loss of the originally-formed water-soluble nitrogen. The total extract of the muscle is made up to a definite volume and made neutral to litmus paper with tenth normal sodium hydroxide.

Cook's method is to weigh 200 grms. in a 450 Erlenmeyer flask, to add 250 c.c. of water and agitate for three hours in a shaking machine. The material is then filtered through linen bags, vigorously and repeatedly immersed with the hands in successive portions of water, pressing out after each extraction until negative biuret reaction results; 2200 to 2500 c.c. of water are generally necessary for this operation, and a small quantity of phenol or thymol should be added.

Weber employs Cook's method at room temperature and with ice water when examining samples of fresh and storage meat, also samples which he had kept for varying lengths of time in his laboratory. A larger amount of soluble proteins resulted when he worked at room temperature. It has not been stated whether this was due to the greater extracting power of water at room temperature, or to greater enzymic action whilst the extracting process was being carried out.

Determination of Collagen.—Place the insoluble proteins, obtained by the above-mentioned directions, in a beaker, add water and heat to boiling for some minutes.

Separate by filtration, wash with boiling water. Deduct the nitrogen of the residue insoluble in boiling water from the nitrogen insoluble in cold water, and multiply by 5.55 for the percentage of collagen. There are drawbacks to this method on account of the difficulty experienced in rendering collagen soluble and the tendency towards decomposition of the protein.

Determination of Coagulable Proteins.—Heat the entire filtrate (or an aliquot portion from the determination of nitrogenous bodies insoluble in water) sufficiently to coagulate the coagulable proteins, filter, wash the insoluble material with hot water, and transfer the filter and contents to a Kjeldahl flask, and determine the nitrogen by Gunning's method, multiply the percentage of nitrogen by 6.25, which gives the percentage of coagulable proteins.

The amount of heating required to obtain maximum coagulation varies with different materials. The Association of Official Agricultural Chemists of the United States directs that the solution should be almost neutralized, but left still slightly acid, and boiled until the globulins are coagulated.

Pennington, experimenting with chickens, evaporates 350 c.c. to a volume of about 100 c.c. before filtering. Grindley and Emmett use 200 c.c. of the solution, add alkali till neutral to litmus paper, and evaporate to 50 c.c. Trowbridge and Grindley, in a later paper, report maximum results from the cold water extract of fresh beef by neutralizing one-fourth of the acidity to phenol-phthalein before coagulation.

Determination of Proteoses, Peptones and Meat Bases.—Dilute the filtrate from coagulated proteins with wash water, concentrate by evaporation, and make up to 100 c.c. The proteoses can be determined by saturating an aliquot portion of the filtrate with zinc sulphate which precipitates the proteoses. The nitrogen found in the precipitate should be multiplied by 6.25, and the meat bases determined by Sjerner's method, as modified by Bigelow and Cook. (See under Meat Extract). To determine peptones deduct from the total nitrogen, the sum of the nitrogen occurring in insoluble nitrogenous bodies, coagulable proteins, meat bases and proteoses.

Determination of Gelatin (modified Stutzer's method).—Thoroughly extract say 10 grms. of the sample with boiling water, then place the extract in a porcelain dish containing about 20 grms. of previously ignited sand and evaporate to dryness. Stir the residue with four successive portions of absolute alcohol using about 50 c.c. each time, and pouring it off through a filter made up of a layer of asbestos fibre on a perforated porcelain plate inside a funnel. Pack the funnel round with chopped ice and arrange it so that gentle suction may be used to help on the filtration. Repeatedly stir the residue with successive portions of about 100 c.c. each of a mixture containing 100 c.c. of 95 per cent alcohol, 300 grms. of ice and 600 grms. of cold water, passing each portion through the asbestos filter. Continue the washing until the solution issuing from the filter is colourless, always keeping the temperature below 5°. Transfer the asbestos with the washed residue to a beaker and thoroughly extract the whole with boiling water. Evaporate the hot-water extract to a small volume, wash into a Kjeldahl flask, in which evaporate to dryness and determine the nitrogen by the Gunning method; $N \times 5.55 =$ gelatin.

Detection of Parasites.—The two principal parasites which are found in sausages are *Trichina spiralis* (fig. 39B) and *Cysticercus cellulosæ* (fig. 39A), the latter being the cause of "measles" in pork. The importance of the absence of these parasites is obvious, since the former is responsible for the disease known as trichinosis, whilst the latter is the larva of *Taenia colium*, a common tape-worm, whose principal host is man. Other forms of *Cysticercus* are found, which are the larvæ of other tape-worms. For their detection, the fat should be removed by a mixture of two parts ether and 1 part alcohol. Schmidt treats the residue (from which pieces obviously not meat may be removed by a needle) with ten times its weight of water containing 0.5 per cent of HCl, and a little pepsin. The mixture is allowed to stand for six hours at 40°. The flesh is thus dissolved, the fat floats on the surface, and the parasites sink to the bottom of the liquid. If the digestion be performed in a separator, the deposited parasites can be run off in a few drops of liquid and examined under the microscope. The *Trichinæ* are easily recognized as thread-like worms coiled in flat spirals, whilst the *Cysticerci* have tape-worm heads and bladder-like tails. The parasites will survive nearly any treatment, except exposure to boiling water temperature.

Detection of Horse-flesh.—Horse-flesh is a common constituent of

continental sausages, some of which find their way to this country. In England a heavy penalty attaches to the sale of horse-flesh without declaring it, so that it is very rarely to be found in English sausages. The detection of horse-flesh, especially when in the minced state, in admixture with other meats is a matter of considerable difficulty, and is often impossible. Much stress has been laid on the presence of a considerable amount of glycogen in horse-flesh, but the methods of de-

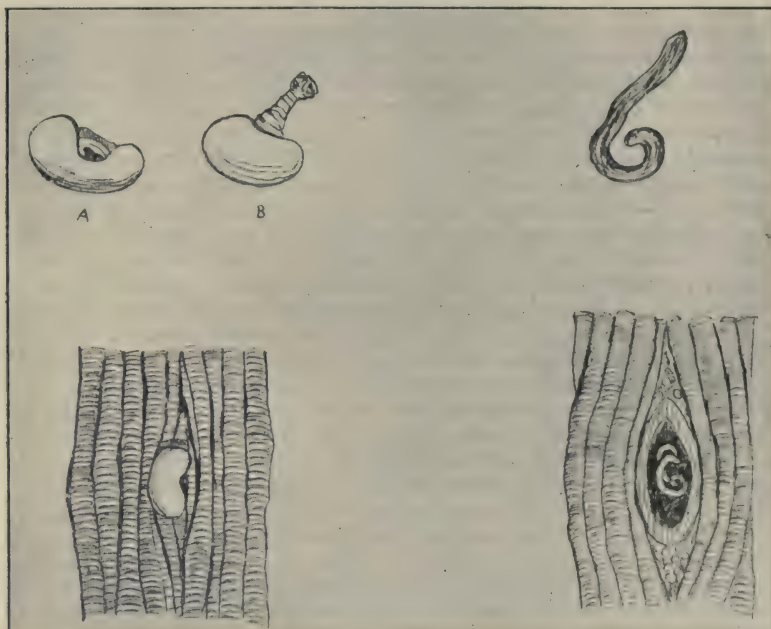


FIG. 39A. — *Cysticercus cellulosae*.
Free (A) with head withdrawn $\times 10$;
(B) with head protruding $\times 10$; and
imbedded in muscular tissue $\times 5$.

FIG. 39B. — *Trichina spiralis*.
Free $\times 100$; imbedded $\times 50$.

tecting and determining this body are of sufficient uncertainty to render them unreliable except in certain well-defined cases.

Glycogen ($C_6H_{10}O_5$)_n was discovered by Bernard in 1857 and has been termed "animal starch". It is found in the livers of many animals in which it is probably stored as a reserve material in times of fasting. It is hydrolysed by ferments into maltose, and by dilute acids direct into glucose. It is coloured red with iodine, a reaction differentiating it from the ordinary starches.

Many processes have been published for the determination of glycogen, but before describing any of these, the following figures due to Bujard ("Forsch. Bericht," 1897, iv. 47) should be examined in

order to indicate the danger of drawing any strong inferences from the results of glycogen determinations:—

	Water.	Per cent Glycogen.	Per cent Glycogen on dried substance.
	Per cent		
Horse-flesh	74.44	0.440	1.72
"	74.87	0.600	2.39
"	76.17	1.827	7.69
"	76.00	0.592	2.475
"	61.83	0.346	2.24
"	72.90	0.174	0.64
"	70.47	1.366	4.62
"	71.84	0.59	2.09
" (smoked)	43.00	0.108	0.19
Beef	73.62	0.206	0.74
"	75.55	0.018	0.073
Veal	76.12	0.346	1.44
"	74.47	0.066	0.25
Pork	54.05	trace	trace
"	61.29	"	"
Pork sausage	67.25	0.240	0.73
Horse sausage	70.04	0.504	1.68
" liver sausage	67.00	1.762	5.34

From these results it appears that the presence of glycogen cannot be considered as definitely indicating the presence of horse-flesh, but that the presence of quantities much over 1 per cent (on the dried substance) would indicate its presence if corroborated by other results, unless much liver were present. If more than 2 per cent (on the dried substance) be present, the presence of horse-flesh is very probable.

Braütigam and Edelmann ("Chem. Central," 1894, 1, 485) give the following quantitative test for the detection of glycogen: 50 grms. of the finely divided flesh are boiled for an hour with four times its volume of water, and dilute nitric acid added to the strained liquid after cooling. Proteids are precipitated and the liquid is partially decolorized. The filtrate is then tested by gently pouring a saturated aqueous solution of iodine on to its surface. In the presence of glycogen a wine-red ring is formed at the point of contact. If the colour is not decided, the flesh may be heated on the water bath with 3 per cent of its weight of KOH dissolved in water, until the muscular tissue is dissolved. The strained liquid is evaporated to half its volume, the proteids precipitated by HNO_3 and the iodine solution added as described above.

Piettre ("Anal. Chem. Anal." 1909, 14, 206) estimates glycogen by boiling 25 grms. of the sausage under a reflux condenser with 80 c.c. to 90 c.c. of an alcoholic solution of KOH (aqueous solution of specific gravity 1.3 diluted with four times its volume of absolute alcohol). The insoluble residue is collected on a filter, washed with hot 80 per cent alcohol, and then with cold alcohol rendered slightly acid with HCl, until all the alkali is removed. The residue is then

heated with slightly alkaline water which dissolves both starch and glycogen. An equal volume of water is then added, thus precipitating the starch. This is filtered off and washed with 50 per cent alcohol; the filtrate is concentrated to a small volume and absolute alcohol added to precipitate the glycogen, which is collected, washed with alcohol, dried and weighed.

Considering the uncertainty attaching to this reaction, further details of other but similar processes are unnecessary.

Niehl gives the following method for the quantitative determination of glycogen:—

The flesh is heated on the water bath for six hours to eight hours with 3 per cent to 4 per cent of its weight of KOH, and four times its volume of water. The liquid thus obtained is evaporated to half its bulk, and HCl, and a solution of mercuric-potassium iodine, added to the liquid when cold, in order to precipitate nitrogenous matter.

The clear filtrate is mixed with 2.5 times its volume of 90 per cent alcohol, and the precipitated glycogen collected on a filter, washed successively with 60 per cent, 90 per cent, and absolute alcohol, and then with ether, and finally with absolute alcohol, dried at 110° and weighed.

Mayrhofer's method consists of dissolving the flesh in aqueous solution of KOH, precipitating proteids by adding HCl and Nessler's reagent, and then precipitating the glycogen with alcohol, and washing it on a tared filter with alcohol and ether and then drying and weighing.

The differences between the fat from the horse and that from other animals have been discussed above (p. 387).

Perhaps the best indications are those given by the examination of the intra-muscular fat and its liquid fatty acids as to the amount of iodine they absorb. Bremer ("Forsch. Ber." 1897, iv. 1) recommends the following process: All visible fat is mechanically removed, and the remaining meat, finely divided, is heated for an hour on the water bath with water. The fat rising to the surface is poured off with the water, and the flesh after several washings with hot water is dried at 100° for twelve hours and extracted with petroleum ether. The fat so obtained is saponified, excess of alkali neutralized with acetic acid and the alcohol evaporated on the water bath. The soap is dissolved in hot water, and hot solution of zinc acetate added. The precipitated zinc soap is washed with hot water and alcohol, dried, and extracted with ether in a Soxhlet. The ether is shaken with dilute sulphuric acid, to decompose the zinc salts of the liquid fatty acids, and then washed three times with water. The ether is then evaporated and the liquid fatty acids are dried at 100°. The iodine value of the fat itself and of the so separated liquid fatty acids, is then determined. On opposite page are tabulated Bremer's results.

When horse-flesh is present the petroleum ether extract has a reddish-brown colour, and the fatty acids also have a slight reddish colour. Bull's flesh gives similar colours, but if this reaction is observed, and the glycogen exceeds 1.5 per cent on the dry substance—or even 1 per cent—and the iodine value of the intra-muscular fat.

exceeds 65 and that of the liquid fatty acids exceeds 95, there is practically no doubt that horse-flesh is present.

	Iodine Values of	
	Intra-muscular Fat. Liquid Fatty Acids.	
	Per cent	Per cent
Horse-flesh sausage	75·8	108·1
" " with about 6 per cent bacon	74·0	104·1
Horse-flesh (brain) and 22 per cent bacon sausage	53·7	92·4
" " " 25 per cent bacon	74·1	102·1
Pork (Thuringian) cervelat with 65 per cent pig's fat	64·3	95·8

For further details as to the presence of horse-flesh in sausages the following papers may be consulted:—

Pflüger and Nerking ("Arch. Ges. Physiol." 1899, **76**, 531).

Mayrhöfer (Forsch. Ber." 1897, iv. 47).

Schütze ("Deutsche Med. Wochs." 1902, **45**, 804).

Colouring Matter in Sausages.—Sausages are very commonly mixed with colouring matter, either with the intention of improving the colour or of concealing a large addition of farinaceous matter. The following details as to the detection of colouring matters apply to preserved foods generally, as well as to sausages:—

The colouring matters usually added are (1) cochineal, (2) aniline colours, (3) iron oxide—often added as Armenian Bole, a form of iron oxide diluted with chalk. There are certain cases where trade usage certainly justifies a small addition of colouring matter. One of these is the colouring of anchovy essences and pastes with a trifling amount of oxide of iron. The preparation is of a colour not acceptable to the public taste, and a little oxide of iron renders it far more inviting, and has been used for many years, and is in every way unobjectionable.

The principal objection to the use of colouring matters in such food stuffs, is that its purpose is to cover the employment of unsound meat, which may be of bad colour. The following methods will reveal the presence of added colouring matters.

The usual colouring matters added to sausages are either some form of oxide of iron or an aniline red. In most cases a small amount of colouring matter is not objectionable, but it is usually necessary to examine the sample in order to decide whether any excess of colouring matter has been added.

Any oxide of iron colour is at once revealed by the examination of the ash, which should at most contain but a trace of iron—say up to 2 per cent of the total ash. Sausages coloured, for example, with Armenian Bole will have a high ash value, and the ash will contain much iron.

Cochineal is sometimes added to sausages. It may be detected by the method described by Klinger and Bujard ("Zeit. Angew. Chem." 1891, 515). The sample, in a fine state of division, is heated with twice its volume of a mixture of equal parts of glycerin and water for three hours on the water bath, the whole being slightly acidified. The yellow solution is poured on to a wet filter, and the colouring matter, if present, is precipitated as a lake by adding alum and ammonia. The precipitate is filtered off and washed, and then dissolved in a small amount of tartaric acid, and the concentrated solution is then examined by the spectroscope against a standard solution of cochineal carmine, when the absorption bands, which should be identical, should be seen, as well-marked bands, lying between *b* and *D*.

Bremer ("Analyst," XXII. 216) has confirmed the utility of this method.

Spaeth ("Pharm. Central." 1897, 38, 884) finds that the artificial colouring matters usually added to sausages can be extracted by warming the finely divided matter with a 5 per cent solution of sodium salicylate on a water bath for a short time. On adding ammonia to the extract, red precipitates are often thrown down, which contain the colouring matter.

Many colouring matters may be extracted by alcohol slightly acidified with hydrochloric acid. A small fragment of white wool is boiled in the liquid, and if it is distinctly dyed, a coal-tar colour is certainly present.

Marpmann ("Zeit. Angew. Mikrosk." 1895, 12) considers that a microscopic examination will reveal the presence of most colouring matters. A section about 1 cm. thick of the sausage is made, and thoroughly moistened with 50 per cent alcohol, and then examined under the microscope. The cell tissue or contents are dyed by most artificial colouring matters, and such dyed cells indicate the presence of added colouring matter. When traces only of a colouring matter have been added, the section may be treated first with xylene, then with carbon tetrachloride, and finally immersed in cedar wood oil and examined under the microscope.

EXTRACT OF MEAT.

Numerous meat preparations exist at the present time, which are prepared in different manners, and which rarely justify the extravagant claims made for them in regard to their nutritive value. One such, claimed in advertisements to be the *most* nutritious of all beef beverages, was found by the author to contain over 70 per cent of mineral matter, principally salt, and no true proteids.

The analyst is called upon frequently to judge the quality of such preparations, but no legal standard can be said to exist for meat extract, hence the rarity with which this class of preparation is dealt with under the Food and Drugs Acts.

Direct adulteration of extract of meat is not common, but the author has had several cases before him in which samples sold as genuine extract of meat contained extract of yeast, an extract which

is now made to closely simulate extract of meat in general characters.

Numerous food products are also on the market under fancy names such as would often lead a person of ordinary intelligence to believe he was dealing with a pure meat preparation, which are in fact little else than extract of yeast containing a small amount of meat extract and various covering flavourings.

The extract of meat with which one is principally concerned analytically is that known as Liebig's Extract of Meat. The name Liebig is not a proprietary one and is open to any one's use, nor is meat extract now made by Liebig's original process.

The best meat extracts to-day consist principally of the portions of the meat, freed from bone and most of the fat, which are soluble in water at a temperature not exceeding 75° C. When the water is used at 100°, the gelatine extracted will be higher than in the former case. When *warm* water is used, the gelatine is low, but albumoses and peptones and the meat bases are present to the full extent, and albumin to a greater or less extent.

It is now generally recognized that extract of meat is rather a food adjunct and a stimulant than a food in the proper sense of the word.

A large number of the analyses quoted below have been made by the author, but for much of the information he is indebted to Allen ("Commercial Organic Analysis," 3rd edition, Vol. IV, pp. 300 *et seq.*).

Many of the extracts of beef of the present day contain added gelatine, meat fibre and peptones. Few if any are made according to the original or modified directions of Liebig. Without wishing to derogate from the admitted value of these preparations, the author cannot help agreeing with the late A. H. Allen in his statement: "It is claimed on behalf of these preparations that the various additions and methods of treatment give them value as real foods, but this is true in but a very limited sense, since the amount of such preparations which would require to be taken to support life is enormously beyond the quantity of any of the preparations which could be consumed without upsetting the system, to say nothing of the extravagant cost of all such preparations if used in quantity necessary to sustain life. In judging of the amount of credence to be attached to statements of the nutritive value and concentration of meat extracts and similar preparations, it should be borne in mind that fresh lean meat contains about 20 per cent of nutritive matter and 75 per cent of water. Hence by the desiccation of 4 lb. of meat there will be obtained 1 lb. of dry substance of which 80 per cent is nutritive proteid matter, the remaining 20 per cent consisting of fat, meat bases, salts, etc. By no possible means can further material concentration of the nutritive matter be effected."

At the same time there are several high class preparations of meat extract to which certain additions have been made, which give to them a true food value, so that such preparations are both stimulants and foods. No standards, other than a requirement of purity, exist in this country for meat extracts or essences, but the following requirements, which have been adopted by the American Association of

Official Agricultural Chemists, are of considerable interest in showing what is expected of a normal preparation in the United States :—

(1) *Meat extract* is the product obtained by extracting fresh meat with boiling water and concentrating the liquid by evaporation, after removal of the fat. It contains at least 75 per cent of total solid matter, of which not more than 27 per cent is ash, and not over 12 per cent sodium chloride. The fat should not exceed 0.6 per cent and the nitrogen be not less than 8 per cent. The nitrogenous compounds contain not less than 40 per cent of meat bases and not less than 10 per cent of creatine and creatinine.

(2) *Fluid extract of meat* differs only from the above in containing not less than 50 per cent of solid matter, and not more than 75 per cent. The proportionate amounts of the other ingredients, after allowing for the extra water, are the same.

(3) *Meat juice* is the fluid portion of muscle fibre, obtained by pressure or otherwise, and may be concentrated by evaporation at a temperature below the coagulating point of the soluble proteins. The solids contain not more than 15 per cent of ash, and not more than 2.5 per cent of sodium chloride; and between 2 and 4 per cent of P_2O_5 ; and not less than 12 per cent of nitrogen. The nitrogenous bodies contain not less than 35 per cent of coagulable proteins and not more than 40 per cent of meat bases.

In judging the value of a meat extract the following are the chief considerations which should be taken into account :—

(1) The amount of water present, (2) the amount of mineral salts, (3) the amount of meat bases, (4) the nature and amount of the proteid matters and other nitrogenous bodies present.

The following tables, for which acknowledgment is made to Mr. Otto Hehner, show the average composition of a number of meat extracts and similar preparations :—

Number.	Description.	Water.		Fat (Petroleum- Ether Extract.)		Gelatine.		Albumin.		Meat-Fibre and Coagulated Al- bumin.		Albumoses.		Peptones.		Meat Bases.		Ash.		Difference.		Sodium Chloride.		Phosphoric Acid.		Total Nitrogen.	
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	Extract of meat.	15.26	0.34	5.18	—	—	—	—	2.12	2.01	8.06	39.32	23.51	4.20	5.81	6.97	9.07	5.81	6.97	9.07	5.81	6.97	9.07	5.81	6.97	9.07	5.81
2	"	15.97	0.21	3.31	—	—	—	—	—	1.75	5.13	41.12	29.36	3.15	9.74	6.76	8.21	9.74	6.76	8.21	9.74	6.76	8.21	9.74	6.76	8.21	9.74
3	"	17.85	0.38	4.56	—	—	—	—	1.81	4.19	10.16	38.90	18.80	2.87	3.31	5.16	9.80	3.31	5.16	9.80	3.31	5.16	9.80	3.31	5.16	9.80	3.31
4	"	22.24	0.29	5.50	—	—	—	—	1.30	3.62	8.44	38.58	20.45	-0.42	5.14	5.50	9.19	5.14	5.50	9.19	5.14	5.50	9.19	5.14	5.50	9.19	5.14
5	Meat juice	55.48	0.10	0.69	1.00	—	—	—	—	—	2.50	12.50	11.06	(15.61 (Glycerin)	4.43	1.52	2.84	4.43	1.52	2.84	4.43	1.52	2.84	4.43	1.52	2.84	4.43
6	"	55.53	0.10	0.75	0.25	—	—	—	—	—	2.87	12.48	12.01	14.01	2.35	2.85	2.92	2.35	2.85	2.92	2.35	2.85	2.92	2.35	2.85	2.92	2.35
7	"	61.61	0.08	1.12	5.62	—	—	—	—	—	1.86	9.44	14.78	4.41	6.96	3.01	3.06	6.96	3.01	3.06	6.96	3.01	3.06	6.96	3.01	3.06	6.96
8	Bouillon	36.19	0.25	1.37	—	—	—	—	4.00	1.16	11.09	24.25	17.93	3.76	6.09	3.58	6.70	6.09	3.58	6.70	6.09	3.58	6.70	6.09	3.58	6.70	6.09
9	Meat juice.	70.19	0.32	0.45	16.44	—	—	—	0.37	0.05	0.37	2.82	6.65	2.34	5.11	0.37	3.28	5.11	0.37	3.28	5.11	0.37	3.28	5.11	0.37	3.28	5.11
10	Essence of beef.	89.68	0.06	5.12	—	—	—	—	—	—	0.57	3.43	1.00	-0.05	0.33	0.40	1.49	0.33	0.40	1.49	0.33	0.40	1.49	0.33	0.40	1.49	0.33
11	Fluid beef.	28.34	1.02	3.81	—	—	—	—	5.37	8.38	13.18	19.38	17.67	2.85	9.07	4.05	8.02	9.07	4.05	8.02	9.07	4.05	8.02	9.07	4.05	8.02	9.07
12	"	44.75	0.62	1.06	—	—	—	—	7.31	2.38	6.25	17.12	19.90	0.61	11.42	3.34	5.46	11.42	3.34	5.46	11.42	3.34	5.46	11.42	3.34	5.46	11.42
13	A proprietary brand	24.34	1.07	4.56	—	—	—	—	5.87	5.56	6.44	34.07	16.50	1.59	5.23	3.35	9.20	5.23	3.35	9.20	5.23	3.35	9.20	5.23	3.35	9.20	5.23
14	" (invalid)	17.47	0.51	2.56	4.43	—	—	—	15.25	1.06	8.82	31.89	16.30	1.91	2.46	1.43	10.21	2.46	1.43	10.21	2.46	1.43	10.21	2.46	1.43	10.21	2.46
15	Meat juice	48.46	0.11	0.25	2.19	—	—	—	0.94	3.65	0.98	11.30	9.95	(22.17 (Glycerin)	4.43	0.62	3.09	4.43	0.62	3.09	4.43	0.62	3.09	4.43	0.62	3.09	4.43
16	Meat and vegetable extract	30.03	0.10	1.69	6.12	—	—	—	—	—	4.85	16.97	23.47	(15.05 (Carbo- hydrate)	11.56	3.02	5.02	11.56	3.02	5.02	11.56	3.02	5.02	11.56	3.02	5.02	11.56

The following are the amounts of nitrogen existing in different forms in the same samples :—

Number.	Description.	Nitrogen existing as—						
		Gelatin.	Albumin.	Meat Fibre and Coagulated Albumin.	Allanoses.	Peptones.	Meat Bases.	Total Nitrogen.
1		0.83	—	0.34	0.32	1.29	6.29	9.07
2		0.53	—	—	0.28	0.82	6.58	8.21
3		0.73	—	0.29	0.67	1.69	6.42	9.80
4		0.88	—	0.21	0.58	1.35	6.17	9.19
5		0.11	0.16	—	0.17	0.40	2.00	2.84
6		0.12	0.04	—	0.32	0.46	1.98	2.92
7		0.18	0.90	—	0.17	0.30	1.51	3.06
8		0.22	—	0.64	0.19	1.77	3.88	6.70
9		0.07	2.63	0.06	0.01	0.06	0.43	3.28
10		0.82	—	—	0.03	0.09	0.55	1.49
11		0.61	—	0.86	1.34	2.11	3.10	8.02
12		0.17	—	1.17	0.38	1.00	2.74	5.46
13		0.73	—	0.94	0.89	1.03	5.61	9.20
14		0.41	0.71	2.44	0.17	1.41	5.07	10.21
15		0.04	0.35	0.15	0.58	0.16	1.81	3.09
16		0.27	0.98	—	0.28	0.77	2.72	5.02

Nos. 1 to 4 represent concentrated beef extracts; 5 to 10 represent specimens of the meat juice type; and 11 to 16 meat preparations containing added matter such as meat fibre, seasoning, etc.

Bigelow and Cook give the following analyses ("U. S. Dept. of Agriculture, Bull. 114," p. 19) of meat *juices* prepared by themselves, from which comparison with meat extracts may be made:—

	1.	2.	3.	4.
	Per cent	Per cent	Per cent	Per cent
Water	85.76	86.85	90.65	91.90
Ash	1.53	1.86	1.36	1.29
NaCl	0.12	0.20	0.15	0.19
P ₂ O ₅	0.37	0.31	0.36	0.29
Fat	0.27	0.30	0.19	0.64
Nitrogen (total)	2.08	1.74	1.16	1.09
" (insoluble protein)	0.16	0.29	{ 0.68	0.12
" (coagulable ")	1.37	0.98		0.41
" (proteose)	0.06	0.07		0.07
" (peptone)	0.16	0.11		0.21
" (amido)	0.33	0.29	0.43	0.27

A few preliminary remarks on some of the nitrogenous matters present in extract of meat are necessary.

The meat bases are amongst the most important of the consti-

tments of meat extract, being largely responsible for its stimulating value.

The following bases have been stated to be present in extract of meat, but only a few of them can be said to have been definitely identified:—

		Nitrogen Factor.
Creatinine . . .	$C_4H_7N_3O$	2.69
Creatine . . .	$C_4H_9N_3O_2$	3.12
Neosine . . .	$C_6H_{16}NO$	8.43
Carnitine . . .	$C_7H_{16}NO_3$	11.57
Vitiatine . . .	$C_5H_{14}N_6$	1.88
Histidine . . .	$C_6H_9N_3O_2$	3.69
Methyl guanidine	$C_2H_7N_3$	1.74
Adenine . . .	$C_5H_7NO_2$	6.36
Xanthine . . .	$C_5H_4N_4O_2$	2.71
Xanthocreatinine	$C_5H_{10}N_4O$	2.54
Hypoxanthine . .	$C_5H_7N_3O$	2.44
Carnine . . .	$C_7H_7N_4O_3$	3.50
Leucine . . .	$C_6H_{13}NO_2$	9.36
Tyrosine . . .	$C_9H_{11}NO_3$	12.93

No satisfactory method exists for their determination, and any results are useless unless accompanied by a statement of the method adopted. Their amount is frequently deducted with some degree of approximation by attributing to them all the nitrogen existing over and above that found to exist in other forms. But an examination of the above figures shows the impossibility of fixing an average nitrogen factor, by which to multiply the amount of nitrogen found in order to convert it into meat bases. Hehner prefers the usual albumen factor 6.25. Stutzer adopts the factor 3.12 which is that for creatine. There is no known means of ascertaining the proper factor, therefore it appears to be the easiest plan to return the "meat bases" in the form of an equivalent of nitrogen. An approximate method for the determination of creatinine, however, exists, which will be discussed later.

The Analysis of Meat Extract.—The analysis of meat extract is admittedly somewhat unsatisfactory, but the following scheme will afford the most useful information available.

Water.—The water is not easily driven off from the extract by the ordinary method. From 1.5 grms. to 2 grms. are weighed into a flat-bottomed platinum dish and dissolved in a little distilled water, and a weighed quantity of recently ignited sand added. The pasty mass is dried in a water oven to constant weight, or more rapidly in an air bath at 105°.

Mineral Matter.—Since the addition of ordinary salt is common in meat extracts, an ash burnt to whiteness or anything approaching whiteness has necessarily lost some of its chlorides. The well-burned ash, therefore, of meat extract is usually below the truth and represents the mineral matter less an uncertain amount of chlorides. The chlorides, if it be considered necessary to determine them, should

be estimated by thoroughly extracting the half-burned ash with distilled water. It is customary to return the chlorides as sodium chloride, but as a matter of fact, the *natural* chlorides of the meat extract are principally potassium chloride. Meat extract in its natural condition contains about 0.06 per cent of chlorides calculated as NaCl for every 1 per cent of dry solid matter it contains. Any excess over this amount is to be regarded as added salt.

Total Nitrogen.—The nitrogen should be estimated by treating about 1 gm. of extract (i.e. the ordinary Liebig's extract; up to 5 grms. of liquid preparations may be used) by Kjeldahl's process.

The Separation of the Nitrogen.—It then becomes necessary to determine the nitrogen existing in the different types of nitrogenous compounds present in the extract.

It will here be convenient to describe the determination of nitrogen by moist combustion. To determine nitrogen by this method, decompose the organic matter by digesting with sulphuric acid and an oxidizer, thus driving off the carbon and hydrogen as carbon dioxide and water respectively, and converting the nitrogen into an ammonium salt from which free ammonia NH_3 is liberated later by making alkaline. Distil the ammonia into an acid solution, the value of which is known, and calculate by titrating the excess of acid. The decomposition, in the Kjeldahl process, is brought about by means of a mercury compound, whilst in the Gunning method it is effected by potassium sulphate which forms bisulphate with the acid.

If nitrates are present and neither method in its simplest form is practicable, it is necessary to use a modification.

For the determination of nitrogen in pepper the Gunning-Arnold method is used, as it is impossible to completely decompose the piperin by the usual processes.

The Kjeldahl-Gunning Method.—Reagents:—

Standard alkali solution N/10NaOH.

Pulverized potassium sulphate.

Sulphuric acid, concentrated.

Sodium hydroxide, saturated solution.

Standard acid solution, N/10 H_2SO_4 or HCl.

An indicator, cochineal.

Granulated zinc.

Take a pear-shaped flask with flat or round bottom, and made of fairly thick Jena glass, and digest and distil preferably in the same flask. The following dimensions are suitable: length 29 cm., maximum diameter 10 cm., tapering gradually to a long neck which is 28 mm. in diameter with a flaring edge. Its capacity should be about 550 c.c.

If preferred, a smaller flask of about 250 c.c. and of the same, shape as the one already described may be used for the digestion and an ordinary round-bottomed flask of 500 c.c. capacity for the distillation. Transfer 0.5 gm. to 3.5 grms. of the sample to the digestion flask, add 10 grms. of potassium sulphate and from 15 c.c. to 25 c.c. of concentrated sulphuric acid.

Hold the flask over a flame, gently heating for a few minutes

below the boiling-point of the acid until the frothing ceases, then gradually increase the heat until the acid boils; continue the boiling until the contents are either a pale straw colour or quite colourless. Place a wire gauze between the flask and flame, or better still a triangle or some similar support.

Cool the contents of the flask, and if the digestion has been brought about in the larger flask suitable also for distilling, as mentioned above, add cautiously 300 c.c. of water and sufficient strong sodium hydroxide to make the contents strongly alkaline, using phenolphthalein as an indicator. When, however, a separate flask is used for distillation add the contents of the digestion flask to the water and the alkali. Add also a few pieces of granulated zinc to prevent, by the evolution of the gas, any bumping and sucking back of the distillate. Shake the flask well and connect with the condenser the bottom of which is provided with an adapter dipping below the surface of the standard hydrochloric or sulphuric acid, a measured quantity of which should be contained in the receiving flask. Continue the distillation until all the ammonia has passed over into the acid, which operation should take from about forty-five minutes to an hour and a half. Usually the first 250 c.c. of the distillate contains all the ammonia.

Titrate with standard alkali the excess of acid in the receiving flask, and calculate the amount of nitrogen absorbed as ammonia. Unless the reagents are known to be absolutely pure and free from nitrates and ammonium salts they should be tested by means of a blank experiment with sugar, thus reducing any nitrates present. Allowance should be made for any nitrogen due to impurities.

It is necessary when purchasing sulphuric acid for the determination of nitrogen to obtain that which is "nitrogen-free," as often the so-called chemically pure acid contains a large amount of nitrogen.

Modification of Gunning's Method to Include Nitrates.—Sodium thiosulphate and salicylic acid are used in addition to the reagents employed in the simpler Gunning method. These should be mixed in the proportion of 30 c.c. of concentrated sulphuric acid to 1 gm. of salicylic acid. Add from 30 c.c. to 35 c.c. of the mixture to 0.5 gm. to 3.5 grms. of the substance in the digestion flask. Agitate the flask well and allow it to stand for a few minutes, shaking occasionally. Next add 5 grms. of sodium thiosulphate, then 10 grms. of potassium sulphate. Heat very gently at first, gradually increasing until the frothing has ceased. Continue to heat until the contents have boiled and are colourless. Then proceed as in the Gunning method.

The Kjeldahl Method.—Transfer 1 gm. of the air-dry substance or a corresponding larger amount of a moist or liquid substance, and 0.7 gm. of mercuric oxide (or a similar amount of metallic mercury) to a 550 c.c. Jena flask. Add 20 c.c. of sulphuric acid. Incline the flask over a Bunsen burner, and heat the mixture below boiling-point for five minutes to fifteen minutes or until the frothing ceases, then increase the heat until the mixture boils quickly. Continue the boiling until the liquid has become almost colourless and for half an hour afterwards. Turn the lamp out, place the flask in an upright position,

slowly add potassium permanganate, shaking until the solution becomes a permanent green or purple colour. Cool, then add sufficient saturated sodium hydroxide solution to render the solution alkaline, and lastly a few grains of granulated zinc, shaking the flask well after each addition. Immediately connect with the distillation apparatus and proceed as in the Gunning method.

One of the most commonly employed processes, especially in works using large quantities of extract of meat, is the following which, however, is admittedly a somewhat empirical process. It consists in making a rough differentiation between the greater part of the proteid and gelatinous matter on the one hand, and the meat extractives and salts on the other. Hehner ("Analyst," x. 221) recommends that 2 grms. of the sample should be dissolved in 25 c.c. of water and 50 c.c. of alcohol be added. The precipitate, consisting principally of gelatinoid and proteid matter, is allowed to settle overnight and the clear liquid decanted in the morning. The precipitate with its adherent liquid is dried in a small basin and weighed. The alcohol precipitate thus obtained is usually about 5 per cent to 6 per cent. Much higher results would indicate added gelatine.

Allen recommends the precipitation from an aqueous solution of the meat extract of the proteid and gelatinoid bodies by means of zinc sulphate; then precipitating peptones and similar bodies by means of bromine, leaving the meat bases in solution. For the best available separation of the various nitrogenous constituents he recommends the following scheme of analysis:—

In the fullest possible analysis of a meat extract, an attempt will be made to discriminate between and determine the amount of nitrogen existing in the various forms of meat fibre and insoluble albumin, coagulable albumin, acid-albumin, albumoses, peptones, coagulable gelatin, gelatin-peptones, meat bases, amido-compounds, and ammonia. Such an analysis is necessarily tedious and rarely necessary, but some of the more important of the above determinations can be affected with reasonable ease and accuracy, and are not uncommonly required of the analyst.

In consequence of the uncertainty attaching to the composition of certain of the nitrogenized constituents of meat extracts, it is often convenient to state simply the amounts of nitrogen found to exist in the various forms, and in cases where it is preferred to state the actual amounts of the nitrogenized bodies present, the corresponding amounts of nitrogen should always be given in addition.

Ammoniacal Nitrogen should be determined by distilling the aqueous solution of a known weight of the preparation with barium carbonate, which is preferable to magnesia.

Unaltered Proteids and Meat Fibre.—Bovril and certain allied high class preparations contain finely powdered meat-fibre. This may be detected by treating the meat extract with cold water, and examining the insoluble portion under the microscope. If meat fibre be found, 5 grms. of a dry preparation, 8 grms. to 10 grms. of an extract or 20 grms. to 25 grms. of a fluid preparation should be treated with cold water, the insoluble matter collected on a filter, washed with

cold water, dried at 100° C., and weighed. The weight obtained represents the meat fibre and insoluble matter of the preparation. An alternative and in some respects preferable plan is to treat the moist residue by Kjeldahl's process. The nitrogen found, multiplied by the usual factor, will give the meat fibrin, as distinguished from the crude meat fibre, etc., obtained by weighing the insoluble matter.

Coagulable Albumin can be determined in the filtrate from the insoluble matter, by rendering the liquid distinctly acid with acetic acid, boiling for five minutes, filtering, and determining the nitrogen in the coagulum. Only insignificant amounts of albumin are usually present in meat extracts, but in certain preparations which have received an addition of scale-albumin the amount may be considerable.

Syntonin.—An aliquot portion of the liquid filtered from the coagulable albumin should be further acidulated with acetic acid and tested with potassium ferrocyanide. If any precipitate be formed the liquid should be heated, and if re-solution does not ensue the presence of *acid-albumin* is certain. If found, the remainder of the liquid should be rendered exactly neutral to litmus, the precipitate filtered off and the contained nitrogen determined.

Albumoses and Peptones.—The filtrate from the precipitate of syntonin, or, in the absence of syntonin, the liquid filtered from the coagulable albumin, is saturated with zinc sulphate. Fifty c.c. of the solution, containing from 1 to 2 grms. of solid matter, is freed from insoluble and coagulable matters, and treated with 1 c.c. of dilute sulphuric acid (1 : 4) to prevent the precipitation of zinc phosphate. It is then completely saturated with zinc sulphate at the ordinary temperature, by adding the powdered salt as long as it continues to dissolve on stirring. The precipitate, which will contain any gelatin and all proteids other than peptones, is filtered off and washed with a cold saturated solution of zinc sulphate. The filter and its contents are then transferred to a flask and treated by Kjeldahl's process. The precipitate produced contains all the albumose of the extract together with any gelatin which may be present and any coagulable or insoluble proteids not previously removed; peptones, meat bases, amido-compounds, and ammoniacal salts are not precipitated.

In an aliquot part of the filtrate, peptones may be determined by precipitation with bromine.

A quantity of the solution containing about 1 gm. of the albuminoid matter is diluted with cold water to a volume of about 100 c.c., and treated in a conical beaker with sufficient hydrochloric acid to render the liquid distinctly acid to litmus. Bromine water, is then added in considerable excess, and the liquid stirred vigorously for some time. The yellowish precipitate which separates is at first flocculent, but becomes more viscous on stirring, and finally adheres in great part to the sides of the beaker. When this occurs the liquid is allowed to stand at rest for about half an hour, or until the precipitate has settled. It is then decanted through an asbestos filter.

The precipitate adhering to the sides of the beaker is washed

several times with cold distilled water, the washings being poured through the filter. Occasionally, when the greater part of the bromine has been washed out of the precipitate, the liquid does not filter clear. It is therefore advisable to keep the washings separate from the filtrate, and if necessary, to add bromine or sodium sulphate to the wash-water.

The contents of the filter-tube (including the asbestos, and, if necessary, the glass-wool) are returned to the beaker used for the precipitation, 20 c.c. of strong sulphuric acid added, and the beaker covered with a watch glass and heated over wire gauze. The substance chars and bromine vapour is evolved. When frothing has ceased, about 10 grms. of powdered potassium sulphate should be added, and the liquid boiled vigorously until colourless. It is then allowed to cool, diluted with water, an excess of caustic soda added, the ammonia distilled off into a known volume of standard acid. From the nitrogen found the amount of peptones present is deduced by multiplying by 6.25.

Ammonia is estimated by distillation with barium carbonate; and total nitrogen by Kjeldahl's process. These two latter determinations are, however, preferably made on a filtered aqueous solution of the original sample. The difference between the total nitrogen and that found in other forms is regarded as existing as meat bases, etc., the actual weight of which is usually calculated by multiplying the nitrogen by the factor 3.12. (But see above).

If the preliminary precipitation with zinc sulphate be omitted the bromine precipitate will include the gelatine, gelatine-peptone, albumen, and similar bodies.

True peptones are present in very small quantity in extract of meat. They may be tested for by the biuret reaction applied as follows: To the aqueous solution, from which gelatine and albumoses have been precipitated by the addition of excess of ammonium sulphate, a few drops of a very dilute solution of copper sulphate are added, and then a large quantity of strong caustic soda solution. A characteristic rose-red colour is produced in the presence of peptones.

König and Bömer hold the following views with respect to the chemical examination of meat extracts and commercial peptones:—

1. Precipitation with 80 per cent alcohol is of no value in determining the form of combination in which nitrogen exists.

2. Albumoses should be determined by salting out with ammonium sulphate or zinc sulphate.

3. The filtrate from the ammonium or zinc sulphate precipitate should be decolorized with animal charcoal, and tested for peptones by the biuret reaction.

4. A determination of the ammonia by distilling an aqueous solution of the extract with ignited magnesia is valuable.

5. When peptone has been proved to be absent, the nitrogen in the phospho-tungstate precipitate, after deducting the nitrogen derived from gelatin, albumoses and ammonia, may be ascribed to the flesh bases. The phospho-tungstate precipitate should stand at least one day before filtration.

6. The difference between the total nitrogen and the sum of the

nitrogen in the forms of gelatin, albumoses, flesh bases, and ammonia gives the amount of nitrogen present in compounds not precipitated by phospho-tungstic acid. No evidence was obtained of the presence of amido or acid amido-compounds.

By the application of these principles to the analysis of typical preparations König and Bömer obtained the following results:—

Nitrogen in the form of—	Liebig's Extract.	
	Per cent of Substance.	Per cent of Total Nitrogen.
1. Soluble albumin	trace	trace
2. Nitrogenous compounds insoluble in 60 to 64 per cent alcohol	0.21	2.26
3. Albumoses	0.96	10.34
4. Peptones	0 to trace	0 to trace
5. Flesh bases	6.81	73.38
6. Ammonia	0.47	5.06
7. Other nitrogenous compounds	0.83	8.96
Total	9.28	—

These amounts of nitrogen represent the following percentages of nitrogenous compounds:—

	Liebig's Extract.	
	Per cent	
1. Soluble albumin	trace	
2. Gelatin and proteids insoluble in 60 to 64 per cent alcohol	1.14	
3. Albumoses	6.05	
4. Peptones	0 to trace	
5. Flesh bases	21.25	
6. Ammonia	0.57	
7. Other nitrogenous matters	5.23	
Total	34.24	

The following appears to be the most reliable scheme for separating the nitrogenous constituents of meat extract. It is largely based on the work of Bigelow and Cook:—

Complete separation of nitrogen compounds involves a discrimination between meat fibre and insoluble protein, coagulable proteins, acid albumin (syntonin), albumoses, peptones, meat bases, gelatin and ammonia.

(1) *Insoluble Proteins*.—Agitate 5 grms. of the extract of the dry,

or 20 grms. to 25 grms. of the fluid variety, with 200 c.c. to 250 c.c. water at about 20° C., and collect the residue on a tared filter. It is not easy to filter such an extract in the ordinary manner, so that a centrifugal apparatus is of considerable use in getting the insoluble matter to settle. After washing the residue, dry at 100°, and weigh, or determine the nitrogen by the Gunning method. Another method is to place the solution in a graduated flask, immerse in plenty of cold water for several hours, frequently shaking. Determine the nitrogen in an aliquot part of the filtrate. Deduct this from total nitrogen and the nitrogen of insoluble proteins is obtained. $N \times 6.25 =$ total insoluble matter including both the meat fibre and insoluble proteins.

(2) *Coagulable Proteins*.—Neutralize the filtrate from (1) exactly to litmus, and add dilute acetic until acidity is just noticeable. Boil for some minutes to make the coagulable proteins insoluble; collect the latter upon a filter (using a centrifuge as recommended above). Determine the nitrogen in the washed residue using the factor 6.25 for coagulable proteins.

(3) *Albumoses or Proteoses*.—Saturate an aliquot part of the filtrate from (2) with zinc sulphate adding the powdered salt as long as it continues to dissolve with stirring and shaking. This precipitates any proteoses, traces of gelatin or insoluble proteins that have evaded being removed but not the peptones or meat bases. Filter, wash, and determine the nitrogen in the residue, using the factor 6.25 for the proteoses, etc.

(4) *Peptones*.—Sjerner's tannin-salt method, modified by Bigelow and Cook. Take an aliquot part of the filtrate from (2) concentrated by evaporation to 20 c.c. or less, in case it is necessary to take more than 20 c.c. and place in a 100 c.c. flask. Add 50 c.c. of a solution containing 30 grms. of sodium chloride, and thoroughly shake the flask to ensure the contents being well mixed with the solution of the sample. Then cool the flask to about 10°. When the solution has reached this temperature add 30 c.c. of a 24 per cent solution of tannin, which must be at the same temperature. The total volume is now at 100 c.c. Again thoroughly mix the contents of the flask, and place it in a cool place and allow it to remain there overnight. In the morning filter the solution at from 8° to 10° into a 50 c.c. graduated flask. Determine the nitrogen in this filtrate, also in an aliquot portion of the filtrate from a blank in which the reagents alone are employed. Multiply the nitrogen found in the 50 c.c. portion by 2 (after correction for the nitrogen in the blank) which gives the total nitrogen in the filtrate, and is calculated to per cent. of nitrogen on the sample used. This includes the nitrogen present as ammonia and all the nitrogen of the meat bases except that portion of the creatin precipitated by the tannin-salt reagent. Add the figure thus obtained to the per cent of nitrogen as determined in (1), (2) and (3). This sum after deduction from the total nitrogen is generally given as the per cent. of nitrogen existing as peptones and is multiplied by 6.25 for the per cent of peptones.

Probably these substances, however, are not true peptones, as the filtrate from (3) usually gives no biuret reaction. It is not unlikely

that they consist chiefly of peptoids, formed by the action of the hot solution on gelatin and polypeptides.

Bigelow and Cook state that the tannin-salt precipitate is not contaminated with other meat bases than creatin. They consider that about one-quarter of the creatin is found in this precipitate. Hence they advise that the percentage of creatin should be determined before and after precipitation with tannin-salt reagent, thus correcting the results obtained. Street considers that this correction is impracticable. He thinks that it is exceedingly difficult if not impossible to completely remove tannin from the filtrate, and that the least trace of tannin prevents the colour reaction for creatin.

(5) *Meat Bases*.—Deduct the per cent of nitrogen found as ammonia in (6) from the per cent of nitrogen found in the filtrate from the tannin-salt precipitate in (4) and multiply the result by 3.12 to obtain the per cent of meat bases.

(6) *Ammonia*.—Dissolve from 5 grms. to 10 grms. of the original sample in a convenient volume of water, add powdered magnesia, then distil. Titrate the distillate and estimate its alkalinity as per cent of NH_3 . Calculate the corresponding percentage of nitrogen also, as it is necessary for determining meat bases in (5).

Determination of Creatin and Creatinin.—An aliquot portion of the filtrate from the insoluble and coagulable protein determination can be used for this determination. This portion, however, must contain sufficient total creatinin after dehydration of the creatin to creatinin to give a reading not far from 8° on the scale of the Dubosc colorimeter, after applying the colorimetric method as outlined by Folin for the determination of creatinin in the urine. Add 5 c.c. of semi-normal hydrochloric acid to this aliquot portion and heat for three and a half hours on a water bath under a reflux condenser. Add 5 c.c. of half-normal sodium hydroxide to neutralize the hydrochloric acid, then add 15 c.c. of a saturated picric acid solution, and 5 c.c. of 10 per cent sodium hydroxide. Agitate the solution and allow it to stand for five minutes; make up to 500 c.c. and compare the colour with a half-normal solution of potassium bichromate in the Dubosc colorimeter. The half-normal bichromate solution corresponds to 10 mg. of creatinin, when the scale is set at 8° , and the amount of creatinin in the aliquot can therefore be estimated without difficulty.

Hegner does not consider this method suitable for application to meat extracts. He concludes that more satisfactory results are obtained from using 25 c.c. of a 1.01 per cent of picric acid with "a quite small amount of alkali". He contends that the precipitate is somewhat soluble in excess of alkali. Emmett and Grindley, who have made an exhaustive study of the method as applied to meats, meat extracts and wines, point out that 15 c.c. of 1.2 per cent picric acid should be used for the original creatinin determination and 30 c.c. for the dehydrated creatinin. They also suggest 5 c.c. of alkali for the original creatinin and 10 c.c. for the dehydrated creatinin, though an additional 5 c.c. does not produce lower results.

Determination of Xanthin Bases.—A true meat extract or meat juice should contain in addition to creatin and creatinin, small quan-

ties of xanthin bases including xanthin, hypo-xanthin, guanin, and adenin. The nuclei of the cells produce these bodies, hence a certain amount of the latter should be obtained in an extract that is prepared from fresh unaltered beef, as well as salts and other extractive matter. The determination of the xanthin bases is consequently valuable in determining the origin of an alleged extract of meat. They are determined by the following method:—

Schittenhelm's Method Modified by Cook.—Take an amount of the standard solution equivalent to 5 grms. of the original extract. Transfer to a large evaporating dish then add 500 c.c. of 1 per cent sulphuric acid. Evaporate to 100 c.c. within four hours to five hours. Cool, and add sodium hydroxide to neutralization, allow to stand overnight, filter, and wash. Treat the precipitate held in suspension in the water with sodium sulphide and warm on the water bath. Add acetic acid to acidify, and filter hot. Add 10 c.c. of 10 per cent hydrochloric acid to the filtrate and evaporate to a volume of about 10 c.c. Filter, make ammoniacal, and add ammoniacal silver nitrate of 3 per cent strength. Allow to stand for several hours, filter the solution, and wash the precipitate with distilled water until it is no longer alkaline. The nitrogen in the precipitate is that of these xanthin bases.

Determination of Gelatin.—This is accomplished by the modified Stutzer method given on page 391.

The recent adulteration of extract of meat with extract of yeast first received attention at the hands of Searl. The following method of detecting this adulteration was published by him, but has been shown to be somewhat unreliable, unless large quantities of the adulterant are present, when by a comparison with genuine extract useful deductions can be drawn.

Make a modified Fehling's solution by dissolving 200 grains of sulphate of copper and 250 grains neutral sodium tartrate in 4 oz. water; add to this 250 grains caustic soda dissolved in 4 oz. of water. Dissolve 10 grains of the sample to be examined in $1\frac{1}{2}$ oz. water, and add to it half volume of the above solution, and boil for a minute or two.

With genuine meat extract no precipitation occurs, but with yeast extract a bulky, curdled precipitate of a bluish-white colour is thrown out, which is almost insoluble in water. When collected, washed, dried, and weighed, several samples of yeast extract have been found to give approximately 1 grain of this precipitate (it looks to the eye more like 20 grains) from 10 grains of extract. It naturally varies a little, according to the amount of moisture and ash contained in the sample. Only one sample of yeast extract has yet been found which did not respond to this test, and in that case it readily reduced the copper. Continental extracts of yeast have given the best results with this test. An English make does not respond to it.

Since yeast extract can be manufactured at a nominal cost from brewers' and distillers' waste products, and its physical characters closely resemble meat extract, it forms an excellent material for fraudulent admixture, for which, until now, no simple chemical test has been available.

As has been mentioned above, a method is available for the determination of the creatin and creatinin present in meat extracts. The amount of these bodies, expressed as creatinin, present in normal extract of meat, varies from 4.5 to 6 per cent, and any considerable shortage below the lower limit will indicate either a badly prepared or very gelatinous extract or the presence of yeast extract.

This reaction has been most fully studied by A. C. Chapman ("Analyst," xxxiv. 475). For a full account of the *raison d'être* of the reaction, reference should be made to the original paper. The estimation is carried out in the following manner:—

A 10 per cent solution of the meat extract in distilled water is prepared. Several 10 c.c. quantities of this solution are then transferred to small beakers, and to each 10 c.c. of normal hydrochloric acid are added, after which the beakers are heated in an autoclave for half an hour at 120° C.; the whole of the creatin present is thus converted into creatinin with the minimum amount of decomposition. To the contents of one of these beakers, cooled to 20°, 30 c.c. of a saturated solution of picric acid, and 15 c.c. of a 10 per cent solution of sodium hydroxide are added. After standing for five minutes, the coloured liquid is made up to 500 c.c. This solution is then matched in a Dubosc colorimeter, against 8 mm. of a standard bichromate solution containing 24.54 grms. per litre. The colour of the 8 mm. column of this solution is practically identical with that of 8.1 mm. of a solution containing 10 milligrams of creatinin per 500 c.c. treated with picric acid and alkali. From the reading obtained, it will be easy to dilute a second 10 c.c. of the solution being tested to correspond practically exactly with the standard bichromate, from which the amount of creatinin can be calculated from the details above given. Or a solution of *pure* creatinin of 20 milligrams per litre may be prepared and treated side by side with the sample being tested, and the colours matched in Nessler glasses, from which the amount of creatinin is calculated.

Micko states ("Zeit. Untersuch. Nahr. Genussm." 1910 **19**, 426-434) that although no substance other than creatinin, which yields Jaffé's reaction, is likely to be present in meat extract, etc., it is advisable, especially in the case of extracts of unknown composition, to have some means besides the reaction just mentioned of ascertaining the actual presence or absence of creatinin in a sample and he therefore gives the following process for isolating the base: 10 grms. of meat extract (larger quantities are taken in the case of mixtures of meat and yeast extracts) are dissolved in water, lead acetate is added until a precipitate is no longer formed, and the whole is diluted with water to a volume of 1 litre. After the lapse of several hours the liquid portion is passed through a filter, and the excess of lead is removed by evaporating the filtrate after the addition of hydrochloric acid, and adding alcohol to the residual solution. The dilute alcohol solution, thus freed from lead, is evaporated to dryness, the residue is dissolved in about 100 c.c. of water, the solution is neutralized with sodium hydroxide and then treated with 10 c.c. of 20 per cent sodium bisulphite solution and 10 c.c. of 13 per cent copper

sulphate solution; the mixture is boiled, allowed to cool, filtered, and the precipitate is washed with cold, previously boiled water. The filtrate is acidified with hydrochloric acid and heated to expel sulphurous acid, and the copper is removed as sulphide. The solution is now evaporated and the residue is extracted with alcohol in order to separate the bases, etc., from the large quantity of alkali salts present. The alcoholic solution is evaporated to a syrupy consistency, then acidified with about 50 c.c. of dilute sulphuric acid, and 30 per cent phospho-tungstic acid solution is added in slight excess. After standing for two days the precipitate is collected on a filter, washed with acidified dilute phospho-tungstic acid solution, until free from chlorides and then rinsed into a beaker with hot water. Hot saturated barium hydroxide solution is added to the contents of the beaker in sufficient quantity to render the mixture distinctly alkaline in reaction, the precipitate is collected on a filter, washed with hot water, and the filtrate, after neutralization with sulphuric acid, is evaporated to a syrup. The latter is dissolved in dilute sulphuric acid, again evaporated, and then dissolved in water and evaporated once more. The syrup obtained is dissolved in the least possible quantity of water, hot alcohol is added, and the mixture is placed aside for about twenty-four hours. The alcoholic solution is then decanted and evaporated, the residue is extracted with alcohol, and the solution is separated from the insoluble portion. The first syrupy residue is also extracted a second time with alcohol, and the united alcoholic extracts are now evaporated: the residue is dissolved in 30 c.c. of water, the solution is heated to boiling and rendered alkaline by the addition of lead hydroxide, the whole being then diluted with several times its volume of hot alcohol. After standing for some hours the mixture is filtered, the alcohol is evaporated from the filtrate, and the lead is removed as sulphide. On evaporating the lead-free solution a crystalline mass is obtained which is dissolved in 40 c.c. of 1·2 per cent picric acid solution. Next day the creatinin picrate formed is collected on a filter, the filtrate is evaporated under reduced pressure, the residue is again dissolved in picric acid solution and allowed to crystallize—these operations being repeated until crystals of creatinin picrate are no longer formed. The creatinin picrate thus obtained is heated with dilute hydrochloric acid, and the picric acid is extracted with toluene, the aqueous solution of the creatinin hydrochloride is concentrated, treated with animal charcoal, and evaporated until crystals begin to form. After cooling, the moist crystalline mass is treated with a mixture of one part of acetone with two parts of absolute alcohol, and the insoluble salt is then collected on a filter. A small quantity of creatinin hydrochloride passes into the filtrate, and the latter must be evaporated and the residue once more treated with the acetone-alcohol mixture. The total quantity of crystals is then dried at 100° C. and weighed. The sample of meat extract examined, yielded 4·5 per cent of creatinin hydrochloride. Yeast extracts do not yield any creatinin hydrochloride when examined by this process.

Cook (U. S. Dept. of Agriculture, Circular 62, 1910) states that meat extracts contain from 6 to 8 per cent of ether-soluble material,

whilst yeast extract only contains 1 per cent. On the dry and fat-free extracts, he finds the following differences :—

	Yeast extract. Per cent.	Meat extract. Per cent.
Ash	27·3 to 30·4	18·2 to 24·9
Nitrogen	7·4 „ 7·5	at least 11·5
Phosphoric acid in ash	3·3 „ 3·9	2·6 to 3·4

Boric Acid is sometimes added to meat extracts as a preservative. The presence of such a substance in an article intended for the use of invalids and persons whose digestion is impaired is very undesirable. Boric acid may be detected and determined by the methods employed for milk. A modified process recently proposed by C. Fresenius and Popp ("Analyst," xxii. 282) and applied by them to the examination of sausages, etc., may also be employed for the determination of boric acid in meat extracts. An amount of the extract corresponding to about 3 grms. of dry substance should be concentrated to a syrup, if necessary, and mixed in a mortar with from 40 to 80 grms. of recently ignited sodium sulphate. The mixture is heated in the water-oven for about an hour, and as soon as the mass is dry some more sodium sulphate is added, and the whole reduced to a fine powder. This is digested with 100 c.c. of cold methyl alcohol for twelve hours, with frequent shaking, after which the alcohol is distilled off. As a rule the boric acid passes over completely in one distillation, but it is desirable to extract the residue a second time, using 50 c.c. of methyl alcohol. The distillate is made up to 150 c.c., and 50 c.c. treated with 7·5 c.c. of water and 25 c.c. of pure glycerine. The mixture is titrated with $\frac{N}{20}$ solution of caustic soda (free from carbonate), using phenol-phthalein as an indicator. A pale rose colour indicates the end of the titration. When it appears, some more glycerine should be added, and if the colour is not permanent the titration is continued till that point is attained. The volume of alkali used (in c.c.) multiplied by 0·0031 gives the boric acid, H_3BO_3 (in grms.), in the volume of the distillate titrated. Borates will be dissolved out of the organic matters by the methyl alcohol, but will not pass over with the free boric acid. They may be determined in the usual manner in the methyl alcoholic extract, after evaporation, ignition, etc.

GELATINE.

It will be convenient to here briefly discuss gelatine as it affects the food analyst.

Gelatine is employed to a considerable extent in the manufacture of certain food products, and is sold retail to householders to a considerable extent.

From an industrial point of view the examination of gelatine and glue may be of an exhaustive nature, but for the purposes of an analysis of gelatine used for edible purposes, the examination will be of a more restricted nature.

Gelatine does not occur—at all events to any extent—ready formed in nature, but is a protein-like body resulting from the decomposition of other substances by the action of boiling water or dilute acids.

The composition of gelatine is approximately as follows :—

	Per cent
Carbon	50
Hydrogen	6·5 to 7
Nitrogen	17 „ 19
Oxygen	25

Traces of sulphur are usually present in gelatine—up to 0·5 per cent—but its significance is unknown, and it does not, probably, enter into the constitution of the gelatine molecular complex. Isinglass is a closely related substance, which readily yields either gelatine or a substance nearly indistinguishable from gelatine. It is obtained from the swimming bladder of numerous species of fishes.

The following analyses of edible gelatine and isinglass are due to Tankard :—

	Isinglass.	Gelatine.
	Per cent	Per cent
Moisture	15·05	17·90
Sulphur	0·38	0·17
Nitrogen in precipitate from aqueous solution by ZnSO_4	14·00	13·09
„ $\times 5·42 =$ “gelatine”	75·88	70·95
Total nitrogen	14·56	14·10
Ash	1·30	3·70

The British Pharmacopœia describes gelatine as the air-dried product of the action of boiling water on such animal tissues as skin, tendons, ligaments, and bones. It is required to be free from chondrin, since it is stated to give no precipitate with acids, alum, lead acetate or 5 per cent solution of ferric chloride.

From the food point of view, the following are the essentials of a good gelatine :—

(1) When soaked in cold water for four hours and then made into a jelly by heating with water, it should yield no offensive odour. If it has an objectionable smell, it should at once be rejected as unfit for food.

(2) If in aqueous solution, it yields an appreciable precipitate with the above-named reagents it is to be regarded as of inferior quality as containing excess of chondrin, resulting from the decomposition of hyaline cartilage.

(3) Its ash should contain no heavy metals, such as copper, which is sometimes present to the extent of 1 grain per lb., and not more than 10 parts of iron per 100,000.

(4) It should not contain more than 5 parts of SO_2 per 100,000. This is determined by distilling a 5 per cent solution of the gelatine,

oxidizing the distillate with bromine water and precipitating the SO_3 formed, by BaCl_2 . Excess of SO_2 , due to the use of SO_2 as a bleaching agent may cause action to be set up with the metal of the tins in which the product is frequently packed, and consequent discoloration of the product, due to the formation of metallic sulphides.

CHAPTER VII.

MICROSCOPICAL ANALYSIS.¹

WHILE chemical analysis furnishes the means of determining the chemical composition of foods and drugs, and thus ascertaining their freedom from adulteration, it is often by microscopical analysis alone that the identity and purity of such as are powdered can definitely be determined. The microscopical examination of powdered foods and drugs should, therefore, never be omitted; even with many substances other than powders valuable results may be obtained. It often affords, in a minimum of time and with a minimum of material, information that cannot be obtained by any other known means.

APPARATUS REQUIRED.

For microscopical analysis the following apparatus will be required:—

1. *Microscope*.—This should be capable of magnifying from 50 to 500 diameters, and should possess a revolving nosepiece and a substage condenser. It should be provided with an Abbé-Zeiss camera lucida for sketching, and a separate eyepiece in which an ocular micrometer is permanently fixed; the value of the divisions of the micrometer, when that particular eyepiece is used in conjunction with each objective, should be determined and kept ready for immediate reference. A polarizing apparatus is of service in detecting crystals, and a mechanical stage is useful when preparations have to be thoroughly searched, but neither of these is absolutely necessary.

The best light is that obtained from a north or east window. Direct sunlight is to be avoided; if that is not possible, it should be modified by means of a white blind. As artificial light, a small, inverted, incandescent gas burner with a ground glass globe, or a carbon filament lamp answers well. In no case should the field be more brightly illuminated than is necessary, and both eyes should be kept open during the work.

2. *Centrifuge*.—A small centrifuge is very effective in separating fine powders from liquids, after bleaching or staining, and saves much time; plain centrifuge tubes answer every purpose.

3. *Dissecting Needles*.—Two plain and two glover's needles,

¹ The author desires to acknowledge his indebtedness to Professor Greenish, who has kindly written this chapter.

mounted in handles with screw caps, which hold needles of any size firmly and allow of ready changing.

4. *Glass Dishes*.—Several small hollowed glass blocks, with covers; these are far preferable to watch-glasses.

5. *Slides*.—Glass slides of the usual size and thickness.

6. *Coverslips*.—Three-quarter inch square No. 2 coverslips are the most generally useful; for the highest power No. 3 may be employed.

7. *Reagent Bottles*.—Half-ounce or 6 drachm square bottles with glass peg-stoppers; they are conveniently kept in wooden trays provided with covers.

8. *Razors*.—One solid and one hollow-ground razor.

9. *Elder Pith*.—This may be obtained from opticians or clock-makers.

10. *Glass Plate* with black and white fields.

PREPARATION OF THE MATERIAL.

The preparation of the material for examination is comparatively simple.

Carefully bulk the powder and set aside from 5 to 10 grms. for examination. Sift 1 or 2 grms. of this through a No. 60 sieve. Should any fragments fail to pass through the sieve, examine them with a lens and pick out such as appear suitable for section cutting. Make and examine sections. Then powder a fresh quantity until it all passes through the sieve.

Transfer about 0.1 gm. of this sifted powder to a glass dish, add a drop of water, and triturate with a glass rod until thoroughly mixed. Then add water, drop by drop, until the mixture acquires the consistency of a thin cream; cover, label, and set aside. Make a similar preparation with a mixture of equal volumes of water and glycerin, and a third with solution of chloral hydrate (see list of reagents). Let these preparations stand for about twelve hours. Then mix the water preparation thoroughly with a glass rod, transfer to a slide a small portion of the mixture, which should be so viscous as to ensure the removal of a representative sample, add a small drop of water, mix, and cover with a coverslip, which should be carefully lowered on to the slide so as to avoid the introduction of air bubbles. The quantity of water should be just sufficient to fill the space between the slide and the coverslip; it should not be so large as to allow the coverslip to float, as then the particles of powder will be in more or less constant motion, nor should it be so small as to cause the coverslip to press on the slide, as this pressure is liable to distort delicate tissues. The quantity of powder should be small enough to avoid any overlapping of the particles, but not so small as to make the distance between them unnecessarily great. Practice will soon indicate the correct quantities. It is better to make a fresh preparation than to proceed with the examination of one that is defective.

Make in the same way a preparation in dilute glycerin and one in solution of chloral hydrate with the powders that have been standing in these liquids.

If the powder must be examined without delay, the following more rapid method may be adopted:—

Place a small portion of the powder, about the size of a mustard seed, on a slide, add a small drop of alcohol, allow most of the alcohol to evaporate, add a drop of water, mix and cover with a coverslip. Mount, similarly, a little of the powder in dilute glycerin and in solution of chloral hydrate. The use of alcohol may be omitted if the powder contains much resin, but in this case the particles of tissue may contain numerous air bubbles which seriously interfere with the examination; these may be driven out by gently warming the preparation until a few gas bubbles escape.

EXAMINATION OF THE PREPARATIONS.

1. Water Preparation.—Examine the preparation in water first, using the low power. Observe the colour of the fragments: this often affords valuable information. If the powder is a very fine one, sclerenchymatous cells, bast fibres, and thick-walled hairs may be found intact; they should be examined under the high power and sketched. Fragments of cells and of cell walls will probably be numerous; they should be similarly dealt with. Portions of the epidermis of leaves, held together by the resistant cuticle, may be found. The larger fragments of tissues, which will be particularly numerous in coarse powders, will probably be too opaque, and may be better examined in dilute glycerin, or in solution of chloral hydrate. On the other hand, cell contents, liberated from the cells, may be found in abundance. Examine for the following cell contents and cells:—

(a) *Starch*.—Make a fresh preparation in water. Bring a drop of dilute solution of iodopotassium iodide into contact with the edge of the coverslip, and, under the high or low power, watch it as it penetrates; if necessary, draw the solution under the coverslip by applying a pointed piece of filter paper to the opposite side. The iodine will stain the starch grains deep blue or nearly black. If starch is present, determine, by means of the ocular micrometer, the length of the largest, of the smallest, and of the most frequently occurring grain.

If the powder consists mainly of starch, it may be desirable to remove this and examine the residue (for methods of doing this, see below).

(b) *Oil and Resin*.—Dilute 1 c.c. of tincture of alkanna with an equal volume of water; mount a little of the preparation in the mixture, allow it to stand for half an hour and examine. Oil and resin will be stained red. If much oil is present, it may advantageously be removed by treating the original powder with ether-alcohol or ether, drying, and making fresh water and other preparations from the defatted powder.

(c) *Aleurone Grains*.—As maceration with water disintegrates aleurone grains, a fresh preparation should be made as follows:—

Moisten a little of the powder with alcohol, let it stand till the alcohol has nearly evaporated, and add a drop of solution of picric acid. After two or three minutes, remove the aqueous liquid with filter

paper, add a drop of glycerin, cover and examine. The aleurone grains will be stained yellow; they may contain one or more crystalloids, globoids, or crystals of calcium oxalate; frequently two of these varieties of contents are present, but very rarely all three. Irrigate with very dilute solution of potassium hydroxide; the ground substance and crystalloids will dissolve instantly, leaving the globoids and calcium oxalate.

(d) *Mucilage*.—This, also, is best detected in a fresh preparation:—

Moisten a little of the powder with alcohol, and add a drop of solution of ruthenium red, (see list of reagents). Many, but not all, mucilages will be coloured bright pink. Treat another portion similarly, with solution of corallin-soda; some mucilages will stain pink; if sieve tubes are present the callus plates will acquire the same colour. Treat another portion of the powder with Indian ink diluted with water; mucilage is often readily seen as colourless or nearly colourless masses.

(e) *Lignified Tissue*.—Mix a small portion of the powder with alcoholic solution of phloroglucin, and allow it to stand for a minute or two, covering to avoid evaporation. Then remove any excess of the solution, add a drop of strong hydrochloric acid, cover, and examine. Lignified tissue will be stained bright red.

2. Glycerin Preparation.—Examine next the glycerin preparation, mounting as directed for the water preparation. The larger fragments of tissue now appear clearer, since the refractive power of glycerin more nearly approaches that of cellulose, but this advantage is partly counterbalanced by the loss of delicate details, such as the striations of starch grains, which, for the same reason, become invisible. Hence a glycerin preparation is more suited for the examination of the larger particles, groups of cells, etc., than it is for that of fragments of cell walls, the finer details of which have often to be determined. The longer the preparation is kept the clearer the particles become, but this is not always an advantage.

3. Chloral Hydrate Preparation.—Solution of chloral hydrate (see list of reagents) has a very powerful solvent action, dissolving protoplasm, aleurone grains, colouring matter and, in the course of a few hours, starch. This property, added to its high refractive index, makes it a more powerful clearing agent than glycerin, but at the same time more liable to obliterate delicate markings.

Examine, in this preparation, the larger fragments of tissue and endeavour, by gradually focussing downwards, to determine the characters of the successive layers of which they consist. Groups of bast fibres and of sclerenchymatous cells, fragments of cork and of epidermis are usually very clear, and crystals of calcium oxalate conspicuous. This medium is excellent for giving a rapid, clear survey of the tissues present, the details of which are often better seen in a dilute glycerin or in a water preparation. Hence it is often desirable to return to one of these after having examined the chloral hydrate preparation. It must also be remembered that solution of chloral hydrate may induce a swelling of the cell wall, particularly if the preparation has been warmed.

In addition to the foregoing preparations, which should invariably be made, the following are useful for special purposes:—

4. Potash Preparation.—Mount a little of the powder in an aqueous 10 per cent or 20 per cent solution of potassium hydroxide; warm gently, cool, and examine. Starch will have been gelatinized, the cell walls expanded, and much colouring matter removed, as is the case with solution of chloral hydrate, but the digestion with hot solution of potassium hydroxide loosens the cells, especially parenchymatous cells, to such an extent that they may frequently be separated from one another. Press the coverslip down moderately firmly with the finger and then push it rather sharply along the slide. By this means disintegration of the tissues can often be effected.

5. Oil Preparation.—Mount a little in almond oil or in a mixture of castor oil and alcohol, and examine. Substances soluble in water, or in the other mountants mentioned, may often be detected.

Further treatment of the powder is occasionally desirable.

6. Removal of Starch.—This is advantageous when dealing with powders consisting largely of starch, as it permits of the concentration and ready examination of the tissues present. Mix 5 grms. or less of the powder with 50 c.c. of water, add 2 c.c. of hydrochloric acid (specific gravity 1.16), boil gently for five minutes, and cool. Centrifuge the turbid liquid, pour off the supernatant solution and wash the deposit once with water, again separating by the centrifuge. Add now a few c.c. of solution of chloral hydrate, stir well, centrifuge again, and finally mount in water, dilute glycerin, or chloral hydrate.

7. Bleaching.—Some powders are so dark in colour that it is necessary to bleach them before applying colour reactions. Mix about 0.1 gm. of the powder with 5 c.c. of solution of chlorinated soda (see list of reagents), and allow the mixture to stand, shaking occasionally, until most of the colour has been removed; dilute with an equal volume of water, separate by centrifugation, and wash the deposit several times with water. The bleached powder may be mounted in water or glycerin, or it may be stained with phloroglucin, chlorzinciodine, etc. It may also be double-stained as follows:—

8. Double-staining.—To the deposit, bleached as above described, add 5 c.c. of Cordonnier's double-stain (see list of reagents), allow it to stand for ten minutes, dilute with an equal volume of water, separate by centrifugation and wash several times with water. Then pass it once through 60 per cent alcohol, once through 90 per cent alcohol, twice through absolute alcohol, and twice through xylol, separating each time by the centrifuge. Mix the mass uniformly with a small glass rod, and mount a little in balsam. Oily and fatty powders should be previously defatted.

9. Disintegration by Schulze's Maceration Mixture.—The elements of which sclerenchymatous tissue consists may be separated by maceration with potassium chlorate and nitric acid (Schulze's maceration mixture), which also removes colour, proteid matter, starch, calcium oxalate, and many other of the cell contents, but does not readily attack lignified tissue, and still less readily suberized tissue. Mix about 1 gm. of the powder in a small flask with 5 c.c.

of water, add 10 c.c. of nitric acid (specific gravity 1.42) and 1 grm. of potassium chlorate in crystals. Warm gently until evolution of chlorine commences. When completely bleached, dilute with an equal volume of water, separate by centrifugation, wash with water, centrifuge again, and mount the deposit in water. The sclerenchymatous cells and fibres may be separated from one another by gentle pressure and then examined; they are generally very clear.

10. Mechanical Separation.—Mechanical separation, either by elutriation or by sifting the powder through sieves of varying degrees of fineness, occasionally yields useful results, especially in the detection of certain tissues, cells or cell contents present in small quantity only. Water is the most useful liquid for elutriation. Groups of sclerenchymatous cells and fibres, and calcium oxalate crystals are among the first fragments to subside; these are followed by groups of parenchymatous cells, while fragments of such cells, starch grains and minute cell contents are among the last. Sifting requires a rather large amount of material; the most useful sieves are those with 120, 100, 80 and 60 meshes to the linear inch.

DETERMINATION OF THE ORGAN FROM WHICH THE POWDER IS DERIVED.

The following procedure may be adopted to determine the organ from which a powder is derived:—

(a) Observe the colour of the particles, as seen in dilute glycerin. A green colour indicates leaf, leaf-stalk, herbaceous stem, or, possibly, calyx of a flower. Examine the chloral hydrate preparation. The presence of an epidermis with stomata and polygonal or wavy epidermal cells, of branching veinlets and of palisade tissue or spongy parenchyma indicates a leaf. Elongated, rectangular epidermal cells are probably derived from the midrib, or from an herbaceous stem; fragments consisting of large, elongated, colourless parenchymatous cells point to the former. If pollen grains are found a flower may be suspected, and search should be made for portions of the petal, which will probably be coloured and have a papillose epidermis, and for the characteristic spirally or reticulately thickened cells from the endothecium of the anthers.

(b) If chlorophyll is absent, examine the chloral hydrate preparation for vessels. In the absence of chlorophyll these will indicate the presence of wood, which may be derived from a trunk, a root, or a rhizome. Abundant, irregular fragments consisting of wood fibres with medullary rays crossing them at right angles, accompanied by comparatively little calcium oxalate, indicate a wood. On the other hand, abundant parenchymatous tissue, with starch, oil or other reserve material, indicates a root or rhizome; in this case, sclerenchymatous cells, or bast fibres, isolated, or in more or less regular groups, may be present.

(c) If vessels are absent, stain a bleached preparation (7) with corallin-soda (see list of reagents) and examine for large sieve tubes. These, in conjunction with fragments of cork, and possibly with isolated or grouped bast fibres or sclerenchymatous cells, indicate a bark.

(d) If the powder is free from chlorophyll, large vessels and sieve tubes, it is probably derived from a seed or fruit. Examine for parenchymatous tissue with reserve material, which may be present in the form of starch, oil, cellulose, aleurone grains, etc. Such tissue is commonly found in seeds and these may, of course, form part of a fruit. The presence of an epidermis with more or less distorted stomata, or of much empty parenchymatous tissue, indicates a fruit.

IDENTIFICATION OF THE POWDER.

Having determined the organ from which an unknown powder is derived the next step is its definite identification. This demands considerable skill and experience. It is best effected by comparing the sketches made of the tissues and elements of the powder with illustrations published with the various works dealing with this subject:—

Greenish and Collin, "Anatomical Atlas of Vegetable Powders".

Greenish, "Microscopical Examination of Foods and Drugs".

Winton, "Microscopy of Vegetable Foods".

Schneider, "Powdered Vegetable Drugs".

Vogl, "Die wichtigsten vegetabilischen Nahrungs- und Genussmittel".

Solereider, "Systematic Anatomy of the Dicotyledons".

The identification should invariably be confirmed by powdering the substance indicated, and comparing the two powders under similar conditions.

DETERMINATION OF PURITY.

The microscopical examination of a vegetable powder often has for its object the determination of the purity or otherwise of a powder of given origin. In such case also, comparison of the powder with a specimen of about the same degree of fineness, known to be genuine, and under exactly similar conditions, is absolutely necessary. Hence the gradual accumulation of a set of authentic specimens of the more commonly occurring foods, drugs, spices, etc., has much to recommend it. Care must be taken to interpret correctly the results of the microscopical examination.

The methods by which powders are sophisticated may be classed under the following heads:—

1. Total Substitution.
2. Intentional Addition.
3. Intentional Abstraction.
4. Intentional Abstraction and Addition.

1. *Total Substitution.*—The total substitution of one powder for another is, as a rule, readily detected, although in some cases as, for instance, the substitution of cassia for cinnamon, considerable caution has to be exercised.

2. *Intentional Addition.*—Here, also, care is necessary not to consider as intentional addition isolated particles accidentally or unavoidably present. Drug mills are frequently cleansed with saw-

dust, and an occasional fibre of pine wood is often met with in commercial powders. Only when the quantity is considerable can this be regarded as serious. Many barks have fragments of wood adhering to them; woods contain portions of bark; rhizomes have the lower leaves, or leaf-bases, or portions of the stem attached; leaves are accompanied by stalks, flowers, fruits, etc. The quantity, therefore, in which foreign substances occur must be taken into consideration.

3. *Intentional Abstraction.*—This may be either mechanical or chemical. Mechanical abstraction may be the result of improper sifting, whereby an undue quantity of those elements which resist pulverization may have been separated from those that are easily powdered; such alteration in the composition of the powder may be detected by carefully noting the proportion in which the various elements occur in a genuine powder with that in which they are present in the sample under examination.

Chemical abstraction may take the form of the removal of some of the soluble constituents, or of admixture with a powder that has already been exhausted. In some cases the absence of colouring matter, or of various secretions, etc., or the presence of gelatinized starch grains may indicate that such form of sophistication has been practised, but, in general, this is a point that must be decided by chemical analysis. In fact, in the analysis of powdered foods and drugs, chemical and microscopical analysis should always go hand in hand.

4. *Intentional Abstraction and Addition.*—It occasionally happens that the attempt is made to cloak the removal of active constituents from a drug by adding a foreign material to it. Such sophistication is readily detected.

DETECTION OF INSECT PESTS.

Powders are occasionally prepared from material that has been attacked by insect pests. The most common of these is the larva of the beetle, *Sitodrepa panicea*. As the mature beetle is present in very minute quantity only, a special method of procedure has to be adopted; the following¹ will be found satisfactory:—

Defat 5 grms. of the powder with ether in a Soxhlet; dry the defatted powder and boil it with 100 c.c. of 5 per cent hydrochloric acid for five minutes in a tared flask; add about 150 c.c. of water, allow the powder to settle, and wash once by decantation. For every 35 grms. of water and powder in the flask add 6 c.c. of concentrated sulphuric acid, cool, and then add, in small portions and cooling again if there is any considerable rise in temperature, 10 c.c. of a 1 in 1 aqueous solution of chromic acid. Allow the mixture to stand with occasional agitation for thirty-six hours or longer. Separate the solid particles by centrifugation, wash them with water, alcohol, and ether successively, dry, remove from the tube, and mount in solution of

¹ Greenish and Braithwaite, "Pharmaceutical Journal," Vol. LXXXV, p. 580.

chloral hydrate, or, if permanent preparations are desired, in xylol balsam.

If the powder contains but little that is soluble in ether the treatment with this solvent may be omitted, and similarly that with the hydrochloric acid. As these pests are almost ubiquitous care must be exercised in condemning a powder in which they have been found.

LIST OF THE PRINCIPAL REAGENTS.

The following reagents will be found sufficient for the examination of most powdered foods and drugs:—

Acetic Acid.—The *Acidum aceticum* of the British Pharmacopœia, containing 33 per cent of real acetic acid. It is used for distinguishing between calcium oxalate and calcium carbonate.

Alcohol.—Absolute alcohol is to be preferred, but 90 per cent alcohol answers most purposes; methylated spirit made with wood naphtha may also be used. It is employed for removing air from sections, and for dissolving resin, volatile oil, tannin, etc. Fats, waxes, and, with the exception of castor oil, fixed oils are only sparingly soluble in it.

Alkanet, Tincture of.—

Alkanet root	20 grms.
Alcohol, 90 per cent	100 c.c.

Macerate for a week and filter.

Tincture of alkanet is much used as a staining agent for fats and fixed oils. For this purpose it should be diluted with an equal volume of distilled water immediately before use, and the powder left in contact with it for about half an hour; suberized cell walls will also be stained (compare Soudan glycerine).

Bismarck Brown.—A very dilute, aqueous solution is used to stain elements after maceration with potassium chlorate and nitric acid, by which they are rendered very transparent. A saturated aqueous solution is sometimes used as a stain for mucilage.

Bræmer's Reagent.—

Sodium tungstate	1 grm.
Sodium acetate	2 grms.
Water to make	10 c.c.

Dissolve. This is one of the best reagents for tannin, with which it produces a yellowish-brown precipitate.

Chloral Hydrate, Solution of.—

Chloral hydrate	50 grms.
Water	20 c.c.

Dissolve. The solution dissolves many of the commoner cell contents, and hence is a most valuable clearing agent.

Chloral Iodine.—Solution of chloral hydrate saturated with iodine, a few crystals of which should be kept in it. Useful for the detection of minute starch grains.

Chlorinated Soda, Solution of.—

Chlorinated lime	200 grms.
Distilled water	1750 c.c.

Triturate the chlorinated lime with the water, added gradually, transfer to a stoppered bottle and add

Sodium carbonate	250 grms.
dissolved in	
Distilled water	750 c.c.

Shake together for four days and filter. To the filtrate add a 10 per cent solution of potassium oxalate as long as a precipitate occurs; stand and filter.

The solution, which is used for bleaching, should be kept protected from light.

Chlorzinciodine, Solution of.—

Liquor Zinci Chloridi, B. P.	175 c.c.
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Evaporate to 100 c.c. and add

Potassium iodide	20 grms.
Iodide	0.5 gm.

Dissolve with a gentle heat and add to the hot solution of zinc chloride; let the mixture stand till cool. This reagent colours cellulose cell walls blue or violet, lignified and suberized walls yellow or brown; it also swells starch grains and colours them blue.

Corallin Soda, Solution of.—

Sodium carbonate	30 grms.
Distilled water	70 c.c.

Dissolve. To a little of this solution, add a small fragment of corallin. The reagent should have a bright pink, not wine-red, colour and should be freshly prepared. It is used for the detection of mucilage and sieve tubes.

Double-Stain, Cordonnier's.—

Iodine green (Grübler's)	1 gm.
Chloroform	10 grms.
Alum-carmines	1000 c.c.

Put them into a flask capable of holding 1200 c.c., in the order named. Shake till dissolved and filter.

Make the alum-carmines for the above as follows:—

Mix 1 gm. of carmine with 5 grms. of powdered alum and a little distilled water. Evaporate to dryness at a gentle heat. Allow the residue to stand twenty-four hours, dissolve it in 100 c.c. of cold distilled water and filter.

Ferric Chloride, Solution of.—A 1 per cent aqueous solution of ferric chloride; it is frequently used as a reagent for tannin.

Glycerin.—Pure glycerin of specific gravity 1.260.

Glycerin, Dilute.—Glycerin diluted with an equal volume of distilled water.

Hydrochloric Acid.—Pure hydrochloric acid of specific gravity 1·16.

Iodine Water.—Distilled water saturated with iodine, a few crystals of which should be kept in the solution. Diluted solution of iodopotassium iodide is often used in its place. It is used for the detection of starch and aleurone grains.

Iodopotassium Iodide, Solution of.—

Iodine	2 grms.
Potassium iodide	1 grm.
Distilled water	200 c.c.

Dissolve.

Maceration Mixture, Schulze's.—Potassium chlorate and nitric acid; the strength of the latter may be varied to suit the requirements of the case; an acid of specific gravity 1·3 is very generally useful.

Phloroglucin, Solution of.—

Phloroglucin	1 grm.
Alcohol (90 per cent)	100 c.c.

Dissolve. The solution gradually darkens and loses its power; it should not be kept more than three months. It is used in conjunction with hydrochloric acid for staining lignified cell walls.

Picric Acid.—A saturated aqueous solution is used to stain aleurone grains.

Potash, Solution of.—A 5 per cent aqueous solution of potassium hydroxide. It dissolves starch, proteid matter, tannin, etc., and is largely used as a clearing agent.

Potash, Strong Solution of.—A 20 or even 50 per cent solution is used to induce swelling of collapsed cell walls.

Ruthenium Red, Solution of (in Solution of Lead Acetate).—To a few c.c. of a 10 per cent. aqueous solution of lead acetate, add sufficient ruthenium red to produce a wine-red colour; the solution should be freshly prepared, as it will not keep long. The reagent is extremely useful for the detection of mucilage.

Soudan Glycerin.—

Soudan red III	0·01 grm.
Alcohol (90 per cent)	5·00 c.c.

Dissolve and add glycerine 5·00 c.c.

The reagent colours suberized cell walls red, especially when gently warmed, and hence is used to detect secretion cells (the walls of which are commonly suberized). It also colours fixed and volatile oils.

Sulphuric Acid.—Pure sulphuric acid of specific gravity 1·843.

PART II—DRUGS.

CHAPTER VIII.

CRUDE DRUGS AND CERTAIN GALENICALS.

IN many cases the identification of drugs is a matter of botanical knowledge, and when recognized, the form in which they exist often precludes the possibility of adulteration. It is, in these cases, in the form of powdered drugs, where one meets with sophistication.

It is true, however, that many "drugs" exist which although pure may be practically useless for the purpose to which such drugs are usually put. For example, samples of belladonna root may be met with that contain so little alkaloid, that they may be practically useless.

An estimation of the active principle present is of course necessary in such cases.

In the present section, apart from tables showing the ash content of the principal crude drugs used, only those where adulteration is practicable and probable will be dealt with.

In general the microscopic examination of a powdered drug, together with a determination of its ash content, will afford sufficient information to decide on its purity or otherwise, except in those cases, of course, where a reliable method exists for determining the amount of a given active principle present.

The following table gives the ash limits for the principal drugs: the figures are compiled from a large number of analyses by Umney Moor, and the author. In all cases they are for the crude drug in its original form. It is to be remembered, as pointed out by Umney ("Pharm. Journ." 4, 15, 492), that there is a loss in weight on grinding, especially in the case of such drugs as gum-resins, where volatile oils will be lost, so that a slight allowance must be made for powdered drugs. In the cases of barks, seeds, etc., this rarely amounts to much and the figures here given would stand for most of the powdered drugs. In the case of those containing volatile constituents, due allowance must be made.

It is also to be noted that roots and rhizomes of a fibrous character are apt to have a comparatively large amount of soil adherent to them, and if this is not removed by careful washing, a high ash value follows:—

ASH STANDARDS.

Drug.	Ash Standard of B.P., 1898.	Proposed Ash Standard.
Acaciæ gummi	Not exceeding 4 per cent	Not exceeding 4 per cent
Aconiti radix	Not stated	6 "
Aloe barbadensis	" "	3 "
" socotrina	" "	4 "
Ammoniacum	" "	7.5 "
Amylum	" "	0.5 "
Anethi fructus	" "	8 "
Anisi fructus	" "	8.5 "
Anthemidis flores	" "	6 "
Araroba	" "	10 "
Arnica rhizoma	" "	10 "
Asaætida	Not exceeding 10 per cent	20 "
Aurantii cortex	Not stated	7 "
Belladonnæ radix	" "	8 "
Benzoinum	" "	2 "
Buchu folia	" "	5 "
Calumbæ radix	" "	6 "
Cambogia	Not exceeding 3 per cent	3 "
Cannabis indicia	Not stated	17 "
Cantharis	" "	7 "
Capsici fructus	Not exceeding 6 per cent	7 "
Cardamomi sem	Not exceeding 4 per cent	6.5 "
Carui fructus	Not exceeding 8 per cent	8 "
Caryophyllum	Not stated	7.0 "
Cascaræ sagradæ cort. . . .	" "	5 "
Cascarillæ cortex	" "	10 "
Catechu	Not exceeding 5 per cent	5 "
Chirata	Not stated	6 "
Cimicifugæ rhizoma	" "	10 "
Cinchonæ rubræ cortex	" "	4 "
Cinnamomi cortex	Not exceeding 6 per cent	6 "
Cocæ folia	Not stated	8 "
Coccus	Not exceeding 6 per cent	8 "
Colchici cormus	Not stated	3 "
Colchici semina	" "	5 "
Colocynthis pulpa	Not less than 9 per cent	Not less than 10 per cent
Conii folia	Not stated	Not exceeding 15 "
Conii fructus	" "	7 "
Coriandri fructus	" "	6 "
Crocus	Not exceeding 7 per cent	7 "
Cubebæ fructus	Not stated	7 "
Cuspariæ cortex	" "	9 "
Cusso	" "	7 "
Digitalis folia	" "	10 "
Elaterium	" "	14 "
Ergota	" "	6 "
Eucalypti gummi	" "	0.7 "
Euonymi cortex	" "	10 "

Drug.	Ash Standard of B.P., 1898.	Proposed Ash Standard.
Filix-mas . . .	Not stated	Not exceeding 5 per cent
Fœniculi fructus . . .	" "	10 "
Galbanum . . .	" "	8 "
Galla . . .	" "	3 "
Gelsemii radix . . .	" "	3 "
Gentianæ radix . . .	" "	5 "
Glycyrrhizæ radix . . .	" "	4 "
Granati cortex . . .	" "	15 "
Guaiaci lignum . . .	" "	2 "
Guaiaci resina . . .	" "	4 "
Hæmatoxyli lignum . . .	" "	2 "
Hamamelidis cortex . . .	" "	5 "
Hamamelidis folia . . .	" "	8 "
Hemidesmi radix . . .	" "	4 "
Hydrastis rhizoma . . .	" "	10 "
Hyoscyami folia . . .	" "	12 "
Ipecacuanhæ radix . . .	" "	5 "
Jaborandi folia . . .	" "	7 "
Jalapa . . .	" "	6·5 "
Kino . . .	" "	2 "
Krameris triandræ radix . . .	" "	2 "
Krameris argenteæ radix . . .	" "	2 "
Limonis cortex . . .	" "	5 "
Linum . . .	Not exceeding 5 per cent	5 "
Lobelia . . .	Not stated	12 "
Lupulinum . . .	Not exceeding 12 per cent	14 "
Lupulus . . .	Not stated	7 "
Mezerei cortex . . .	" "	4 "
Moschus . . .	Not exceeding 8 per cent	8 "
Myristica . . .	Not stated	4 "
Myrrha . . .	" "	8 "
Nux vomica . . .	" "	2 "
Opium . . .	" "	5 "
Papaveris capsulæ . . .	" "	10 "
Pareiræ radix . . .	" "	4 "
Physostigmatis sem. . .	" "	4 "
Pimenta . . .	" "	6 "
Piper nigrum . . .	" "	7 "
Pix burgundica . . .	" "	1 "
Podophylli rhizoma . . .	" "	5 "
Pruni virginianæ cortex . . .	" "	6 "
Pterocarpi lignum . . .	" "	1 "
Pyrethri radix . . .	" "	5 "
Quassia lignum . . .	" "	4 "
Quillaia cortex . . .	" "	12 "
Rhei radix . . .	" "	12 "
Rhœados petala . . .	" "	16 "
Rosæ gallicæ petala . . .	" "	4 "
Saccharum lactis . . .	Not exceeding 0·25 per cent	0·25 "
Sambuci flores . . .	Not stated	10 "

Drug.	Ash Standard of B.P., 1898.	Proposed Ash Standard.
Sarsæ radix . . .	Not stated	Not exceeding 8 "
Sassafras radix . .	" "	2 "
Scammoniæ radix . .	" "	12 "
Scilla . . .	" "	4 "
Scoparii cacumina . .	" "	4 "
Senegæ radix . . .	" "	5 "
Senna alexandrina . .	" "	14 "
Senna indica . . .	" "	14 "
Serpentariæ rhizoma . .	" "	10 "
Sinapis . . .	" "	5 "
Staphisagriæ sem. . .	" "	15 "
Strophanthi sem. . .	" "	5 "
Stramonii folia . . .	" "	15 "
Stramonii sem. . .	" "	3 "
Styrax præparatus . .	" "	0.5 "
Sambul radix . . .	" "	6 "
Taraxaci radix . . .	" "	7 "
Tragacantha . . .	" "	4 "
Uvæ ursi folia . . .	" "	4 "
Valerianæ rhizoma . .	" "	10 "
Zingiber . . .	" "	5 "

ACACIA.

Gum acacia is officially described as a gummy exudation from the stem and branches of *Acacia Senegal* and other species of *Acacia*.

It is described as being insoluble in alcohol, entirely soluble in water, yielding a feebly acid solution. When dissolved in an equal weight of water, the solution should neither form a glairy mucilage, nor, after admixture with more water, should it give a gummy deposit on standing. An aqueous solution forms an opaque jelly with lead subacetate solution, and with borax solution a more or less translucent white jelly. Its solution gives no precipitate with lead acetate solution, and is not coloured blue or brown by a small quantity of iodine solution (absence of starch and dextrin), nor bluish-black by ferric chloride solution. It does not reduce Fehling's solution, nor yield more than 4 per cent of ash.

Gum acacia, or gum arabic, as it is also frequently termed, consists principally of the calcium salt of arabic acid, which is also present in combination with traces of magnesium and potassium. The formula for arabic acid is uncertain, but O Sullivan considers it to be $C_{80}H_{142}O_{74}$ and that of its calcium salt, $C_{80}H_{142}O_{74}CaO$.

Arabic acid is obtained by dialysing an acidified solution of the gum, the colloidal solution remaining in the dialyser being lævorotatory.

Gum acacia should have the properties ascribed to it by the Pharmacopœia, as mentioned above. It should contain from 10 to 13 per cent of water. Its solution should not yield more than a slight precipitate with solution of mercuric chloride.

According to Palladino, dextrin may be detected as follows:—

If an alkaline solution of gum is boiled for a minute with aniline sulphate, the liquid remains pale yellow with a greenish tinge in the absence of dextrin, but becomes orange-yellow or brownish-red if dextrin is present. Other results are given in the following table, in which (1) represents the specific gravity at 15° of solutions containing 13·024 grms. of the gum in 100 c.c.; (2) is the viscosity of the same solution as compared with water; (3) is the acidity in terms of arabic acid; (4) is the specific rotation, $[\alpha]_D$, at 16°.

	1.	2.	3.	4.
Kordofan	1·0450	1·4166	6·29	- 26·47
Galam	1·0448	1·3333	7·23	+ 2·11
Salabreda	1·0448	1·4166	8·18	+ 14·57
Bas du Fleuve	1·0450	1·5000	6·92	- 28·47
Arabic (Kordofan)	1·0454	1·3333	6·92	- 23·02
Zula	1·0448	1·1666	7·23	+ 12·84
Ghoziri	1·0446	1·3333	9·75	+ 45·01
Amrad	1·0425	1·3333	5·03	+ 71·81
Australia	1·0438	1·1666	5·03	+ 62·21
Cape	1·0395	1·5000	7·86	+ 33·09
Suakim	1·0450	1·3333	10·06	- 21·17
Turique	1·0450	1·5833	9·12	+ 34·41
Geddah	1·0449	1·4166	5·34	- 24·87

Guichard has examined the rotatory powers of the various acacia gums in the market, and finds that they form three series: those of Galam, Mogador, and Australia have a rotatory power near +16°; Arabic, Aden, and Amrad gums border upon +32°, whilst gum Ghatti has a rotatory power close upon +64°. The differences may be explained by the view that the gums are mixtures of several dextro-rotatory and lævorotatory substances.

Inferior gums often contain a trace of reducing sugar, but any notable amount would probably be due to added dextrin which is sometimes added to powdered gum acacia.

Dextrin may be separated by dissolving the sample, concentrating the liquid to a syrup, and precipitating this by means of 10 times its volume of 90 per cent alcohol. One grm. of the dried precipitate is then dissolved in 10 c.c. of water, the solution mixed with 30 c.c. of 60 per cent alcohol, and 4 drops of 25 per cent ferric chloride solution. About 0·2 grm. of powdered chalk is then added and the whole well stirred. The precipitate is washed with 60 per cent alcohol, and the filtered liquid containing the dextrin is mixed with methylated spirit and the precipitated dextrin allowed to stand for twenty-four hours. The liquid is then decanted, the dextrin dissolved in a little water, and the liquid filtered, if necessary, and evaporated, and the residue weighed. To determine the arabin, the ferric chloride and chalk precipitate is dissolved in a slight excess of hydrochloric acid, the arabin precipitated by strong alcohol, washed with alcohol, then dissolved in water, the water evaporated, and the residue weighed.

The following figures were obtained in samples of "gum arabic" from *acacia* and allied plants by the Technical Department of the Imperial Institute :—

	Gum from Borgu.	Gum from Geldam	Gums from Garfung Kano.			Gum from Bornu.	Gum of <i>Acacia cafra</i> from Koutagora.
	Per cent	Per cent	1 Per cent	2 Per cent	3 Per cent	Per cent	Per cent
Moisture	14.5	14.0	17.8	17.8	17.4	15.0	17.7
Ash	2.26	2.9	2.6	2.6	3.2	3.1	2.6
Dry matter (soluble in water)	82.2	86.0	82.0	79.0	78.0	85.0	81.2
Reducing sugars	0.9	—	traces	1.2	traces	nil	—
Acidity (milligrams KHO for 1 gram of gum)	—	1.6	2.0	0.8	0.8	traces	—
Relative viscosity of 10 per cent solution	22.0	21.0	14.2	21.2	22.5	21.8	10
Colour of solution . . .	pale yellow	almost colourless	almost colourless	pale brown	pale brown	pale yellow	almost colourless

Three samples of gum from the Gold Coast Colony were examined, these gums being obtained from *Acacia Sieberiana*, *Burkea Africana*, and *Pseudocedrela Kotchyi*.

Botanical Origin.	<i>Acacia Sieberiana</i> .	<i>Burkea Africana</i> .	<i>Pseudocedrela Kotchyi</i> .
Moisture	14.9	15.06	13.7
Ash	1.8	2.3	2.6
Insoluble matter	7.02	0.9	0.35
Reducing sugars	traces	—	considerable traces
Relative viscosity of 10 per cent solutions	27.0	18.2	10.8
Acidity (milligrams KHO per 1 gram gum)	3.6	3.92	3.1
Colour of solution	yellowish-brown and turbid	dark brown	yellowish-brown and turbid

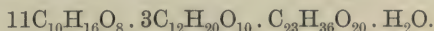
TRAGACANTH.

Tragacanth is officially described as a gummy exudation obtained from *Astragalus gummifer*, and other species, known in commerce as Syrian tragacanth.

It is officially stated that it is sparingly soluble in water, but swells into a gelatinous mass which may be tinged blue or violet with iodine.

There are numerous grades and varieties of tragacanth found in commerce, but it is only the Syrian tragacanth—and only the flattened flakes of that—that is official. Small masses of nondescript shape constitute the “hog” tragacanth of commerce, which may or may not be pure—but is not official.

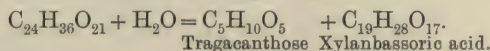
The composition of tragacanth is very complex, but according to O'Sullivan the portion soluble in water consists of a mixture of gum acids. They belong to a series of poly-arabinan-trigalactane-geddlic acids, the chief of them being represented by the formula



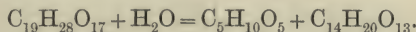
This has a specific rotation $\alpha_D = 88^\circ$.

On hydrolysis these acids yield arabinose and possibly galactose. The insoluble portion consists of an acid body which is termed bassorin.

Bassorin has not been obtained perfectly pure; it is of an acid nature, having the rotation $\alpha_D = +98^\circ$. When treated with excess of alkali it yields two acids, α - and β -tragacanthan-xylan-bassoric acid. The former $C_{24}H_{34}O_{20}H_2O$, is soluble in water, and has a rotation $\alpha_D = +138.6^\circ$; the latter is insoluble in water, and has a rotation $\alpha_D = +163^\circ$ to 164° . Both acids, when hydrolysed with sulphuric acid, yield the same products, *Tragacanthose* and *xylanbassoric acid*. Tragacanthose is lævorotatory, $\alpha_D = -30^\circ$, and is a pentose. Xylanbassoric acid is strongly dextrorotatory, $\alpha_D = +200^\circ$. The hydrolysis is represented by the equation



Xylanbassoric acid is almost insoluble in cold water, but its alkaline salts are soluble; when further hydrolysed it yields bassoric acid and xylose, according to the equation



The last acid is insoluble in water; the optical rotation of its alkaline salts is found to be $\alpha_D = +225^\circ$.

A genuine tragacanth contains about 10 to 15 per cent of moisture, and yields from 2.5 to 4 per cent of ash.

If it be allowed to stand with water for four hours and then heated until a thick solution results and then diluted with a large volume of water, tragacanth forms a ropy liquid which may be passed through a filter, leaving a small amount of starch and cellulose. This liquid should not be precipitated nor form a jelly, with borax solution, sodium silicate or ferric salts.

Powdered acacia is often added to powdered tragacanth as an adulterant. The sample should be rubbed into a cream with water, and then diluted and vigorously shaken for a time, and then the

liquid filtered. In the presence of acacia, a precipitate or jelly results on the addition of borax or sodium silicate solutions.

According to Scoville ("Druggist's Circular," March, 1909) gum tragacanth in the form of powder is liable to be frequently adulterated with the product known as "Indian gum". This gum is generally the product of either *Sterculia urens*, or *Cochlospermum gossypium*. Scoville gives the following table of reactions of genuine tragacanth and of the so-called Indian gum. He states, however, that so far as the detection of the latter in mixtures with the former is concerned, the only characteristic reactions are the acidity of the Indian gum and its behaviour towards borax solution.

Test.	Tragacanth Solution 2 per cent.	Indian Gum Solution 2 per cent.
Appearance of solution.	Opaque, slimy and semi-fluid.	Transparent, non-adhesive jelly.
Reaction to litmus.	Neutral.	Acid.
Iodine test.	Blue colour.	No reaction.
Solution heated with 5 per cent KOH.	Froths on shaking.	No frothing on shaking.
Ferric chloride solution.	Gelatinizes.	Slightly hardens.
Subacetate of lead.	Precipitates in mass.	Precipitates in clots.
Alcohol (equal volume).	Precipitates slowly.	Clear mixture.
Alcohol (two volumes).	Precipitates at once.	Precipitates slowly.
Heated with 2 per cent HCl.	Slight darkening.	Red-brown colour.
Borax solution.	No change in consistency in 3 days.	Becomes slimy and tacky with marked stringing when poured from the vessel.

The acidity test is certainly not very reliable and should be ignored unless the reaction be very markedly acid. The borax test is certainly indicative of adulteration, but the nature of the adulterant is not decided by it. It is best carried out as follows: 2 grms. of powdered gum are shaken with 100 c.c. of water until quite free from lumps. If the powder is first moistened with 3 c.c. of alcohol, and the water added quickly, the semi-solution is prepared more rapidly. Two grms. of powdered borax are then added and the mixture shaken until the borax is dissolved. The mixture is allowed to stand overnight. Pure tragacanth will not have altered, except by a slight darkening in colour. In the presence of the Indian gum, the liquid will have lost its transparency and have become more or less slimy and tacky according to the amount of adulterant present.

AMMONIACUM.

This gum resin is the product of *Dorema ammoniacum* and probably other species, and is official in the Pharmacopœia. 10

The following tests are given: The freshly fractured surface is

coloured yellow by solution of potash, and dark red or orange by solution of sodium hypochlorite. If a small fragment be strongly heated in a test tube, and the contents of the tube, after cooling, boiled with water, the resulting solution when largely diluted with water and rendered alkaline with ammonia does not exhibit a blue fluorescence (absence of galbanum and asafœtida). The last described reaction is due to the characteristic fluorescent nature of umbelliferone (see under galbanum). Ammoniacum of commerce consists of about 60 to 70 per cent of true resinous matter, which is a mixture of esters of ammosinotannol $C_{18}H_{30}O_3$ (in which the salicylic ester is predominant), and of free resin acids. Traces of free salicylic acid and of essential oil are present and from 10 to 25 per cent of gum, which is similar in properties to gum acacia.

The ash of ammoniacum varies from 2·5 to 7·5 per cent, the latter being the highest that should be allowed.

The following are the most reliable analyses of good commercial ammoniacum:—

Constituents.	Plugge.	Buchholz.	Braconnet.	Moss.	Hirschsohn.
	Per cent	Per cent	Per cent	Per cent	Per cent
Ethereal oil . .	1·27	4·0	7·2	2·8	1·43 to 6·68
Moisture . . .	5·10				0·81 „ 3·27
Ash	2·00				2·02 „ 16·88
Resin	65·53	72·0	70·0	68·6	47·12 „ 69·22
Gum	26·10	22·4	18·4	19·3	—
Bassorin . . .	—	1·6	—	—	—
Gelatinous substances . .	—	—	4·4	5·4	—
Extractives . .	—	—	—	1·6	—
Sugar, etc. . .	—	—	—	—	1·61 to 4·59
Per cent sol. in water . . .	—	—	—	—	11·85 „ 25·74
Residue . . .	—	—	—	—	0·81 „ 3·09

A genuine ammoniacum should respond to the reactions of the British Pharmacopœia and should not contain more than 7·5 per cent of mineral matter, nor more than 40 per cent matter insoluble in 90 per cent alcohol. Good samples will often contain as little as 15 per cent to 25 per cent of matter insoluble in alcohol.

The resin extracted by alcohol from ammoniacum should have an acid value between 70 and 100, and an ester value of 50 to 100. These limits are occasionally exceeded.

The gum may be determined by dissolving 2 grms. of the sample in 15 c.c. of a 60 per cent chloral hydrate solution, and filtering this into 100 c.c. of alcohol, which precipitates the gum, which is collected washed with alcohol, dried and weighed.

ARAROA.

This drug, also known as Goa powder, is usually imported in its crude form, not powdered. It usually contains a good deal of woody fibre which is directed by the British Pharmacopœia to be picked out as far as possible. When powdered it forms a brown to umber-coloured powder containing a large proportion of chrysarobin, but no chrysophanic acid.

Most samples of this drug are sold for the purpose of manufacturing chrysarobin, so that the only point of importance to determine is the amount of that acid present. This is often as high as 65 per cent in the crude drug.

No limits for foreign matter are given in the Pharmacopœia, and if a sample contains 50 per cent of chrysarobin it will correspond with the official requirements.

Commercial samples vary in their content of mineral matter very greatly. On an average of thirty samples of parcels as imported into London, the author has found the mineral matter to vary from 7 per cent to 28 per cent (values of 80 per cent given by Pearmain are obviously for grossly adulterated samples), and the chrysophanic acid from 42 per cent to 69 per cent.

In order to determine the ash and chrysarobin, a large amount of the sample should be finely ground, as the size of the fragments of woody tissue are so variable as to make it difficult to get a representative sample. Two grms. should be ignited and the residue weighed.

For the determination of the chrysarobin, 2 grms. to 3 grms. may be extracted with chloroform in a Soxhlet tube. The resulting chrysarobin, obtained by evaporation of the chloroform, should answer to the following characters: it should leave not more than 1 per cent of ash when incinerated, and should be almost entirely soluble in 90 per cent alcohol.

Jowett and Potter find "chrysarobin" to contain the following compounds ("Jour. Chem. Soc." 81, 1575):—

Chrysarobin $C_{15}H_{12}O_3$ is the anthranole of chrysophanic acid and identical with chrysophanoanthrone obtained by the reduction of chrysophanic acid. It melts at $204^{\circ}C$. When acetylated with acetic anhydride alone, a mixture of diacetylchrysarobin (m.p. 193°) and triacetylchrysarobin (m.p. $238^{\circ}C$.) is obtained, but if sodium acetate and acetic anhydride are used, the triacetyl compound is alone produced.

Methyl ether of dichrysarobin $C_{31}H_{26}O_{71}$ melts at $160^{\circ}C$. It yields a soluble pentacetyl compound (m.p. $135^{\circ}C$.), identical with Hesse's hexacetyldichrysarobin.

Dichrysarobin $C_{36}H_{24}O_7$ does not melt below $250^{\circ}C$., but blackens and chars gradually. On acetylation, hexacetyldichrysarobin (m.p. 179° to $181^{\circ}C$.) is obtained.

A substance $C_{17}H_{14}O_4$ (m.p. $181^{\circ}C$.), which yields an acetyl compound (m.p. 215° to $216^{\circ}C$.).

ASAFÆTIDA.

Asafœtida is a gum resin obtained by the incision of the roots of *Ferula fœtida*, and other species.

It contains the following substances: asaresinotannol $C_{24}H_{33}O_4OH$, and its esters; gum; essential oil of a foul-smelling nature; traces of vanillin, and mineral matter and woody fibre.

It is an evil-smelling substance, occurring in masses or in tears—the latter being the purer variety. The British Pharmacopœia requires it to contain not less than 65 per cent of matter soluble in 90 per cent alcohol, and not more than 10 per cent of ash. According to most observers the ash value should be 20 per cent, as the great majority of commercial samples fail to satisfy the Pharmacopœial requirements. However, some samples occurring in tears are practically pure gum resin, and will satisfy the above requirements.

Of twenty samples examined by the author, the ash varied from 16 per cent to 55 per cent, and the amount soluble in 90 per cent alcohol from 31 per cent to 68 per cent. The examination of asafœtida should include the determination of the mineral matter and of the resinous matter extracted by 90 per cent alcohol. The petroleum ether extract should not exceed 7 per cent. The presence of colophony would be indicated by a high solubility value in petroleum ether.

The pure resinous matter extracted with alcohol should have the following characters:—

Acid value	Rarely exceeds 65
Ester value	Rarely below 150

The figures are rather variable, but a high acid and low ester value indicates the presence of colophony; this, however, should be confirmed by the solubility in petroleum ether and by the transient violet coloration produced by carefully pouring a few drops of 50 per cent sulphuric acid on to a solution of the resin in acetic anhydride.

Puckner has published ("Proc. American Pharm. Assn." 1890) the analyses on page 438 which give the characters of the ash of asafœtida. Sample No. 5 was probably grossly adulterated with siliceous matter.

Tincture of Asafœtida is an extract of 4 ounces of the drug with 20 fluid ounces of 70 per cent alcohol. There is obviously no official standard of extractive matter in this tincture, since the 65 per cent of resinous matter, etc., present in the official drug is not necessarily extracted by 70 per cent alcohol. Tinctures made from the drug having the official characters gave the following results on analysis:—

Specific gravity	0.910 to 0.918
Solid residue	9 " 10 per cent
Alcohol (by volume)	60 " 63 "

Commercial samples, being made with low-grade asafœtida, are very frequently below the proper standard of solid matter. It is not clear why this tincture is not made with 90 per cent alcohol.

	Soluble in Alcohol.	Total Ash.	Insoluble Ash (earth, clay, sand, etc.)	Alumina and Iron.	CaO.	MgO.	SO ₂ .	CO ₂ .
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
No. 1 in mass .	59·49	19·45	2·32	1·16	7·12	1·14	8·57	1·09
No. 2 „ .	27·39	56·03	1·37	·42	25·07	2·03	20·49	10·78
No. 3 powdered.	44·48	38·59	2·36	·60	16·57	1·39	16·18	5·32
No. 4 „ .	33·47	47·86	1·95	·60	18·85	·42	23·51	2·62
No. 5 „ .	31·35	55·38	21·96	2·57	9·91	6·49	8·96	4·25

Essential Oil of Asafoetida.—This is a foul-smelling oil, obtained to the extent of 4 per cent to 10 per cent from fair-grade samples of asafoetida. It has a specific gravity ·975 to ·990 and optical rotation about -10° . Semmler has investigated this oil, having separated by fractional distillation under reduced pressure two terpenes, one of which was probably pinene, and a sesquiterpene, which had a lavender-like odour. The remainder of the oil consists chiefly of compounds containing sulphur. According to Brannet, the oil contains allyl sulphide and allyl disulphide, but Semmler denies this. Sulphur compounds of the formulæ $C_7H_{14}S_2$, $C_{10}H_{20}S_2$, $C_8H_{16}S_2$, and $C_{10}H_{18}S_2$ were found, together with an oxygenated body of the formula $C_{10}H_{16}O$, or a multiple of this.

A very minute trace of the oil, or of an alcoholic extract of the gum resin, is present in several of the best-known sauces made in England and America.

BALSAM OF PERU.

This balsam is exuded from the trunk of *Myroxylon Pereira*. The British Pharmacopœia gives the following tests for this drug. It is soluble in chloroform; 1 volume is soluble in 1 volume of 90 per cent alcohol, but on the further addition of 2 or more volumes, the liquid becomes turbid. Specific gravity 1·137 to 1·150, 10 drops rubbed with 0·4 grm. of lime produces a soft mixture (absence of copaiba and “resins” (?)). It should not diminish in volume when shaken with an equal volume of water (absence of alcohol). About 40 per cent of resin should separate on the addition of three times its volume of CS_2 , and the clear supernatant liquid should not have more than a pale brown colour and slight fluorescence (absence of gurgun balsam). Five grms. shaken with 5 c.c. of a solution of NaOH of specific gravity 1·13, and then washed with three successive quantities

of 15 c.c. of ether, and the ether cautiously evaporated, should give a residue weighing from 2.85 grms. to 3 grms. This residue should require from 11.9 c.c. to 12.8 c.c. of normal alkali for saponification (presence of a due proportion of cinnamein).

The principal constituents of Peru balsam are the esters benzyl cinnamate (cinnamein) and cinnamyl cinnamate (styracin). A little free benzyl alcohol is also present, and traces of vanillin and free cinnamic acid. The cinnamic and benzoic esters of an alcohol peruresinotannol $C_{18}H_{20}O_5$ are also present.

Balsam of Peru is a black, viscous liquid and has an aromatic, agreeable odour, and being an expensive product is frequently adulterated. The principal adulterants are colophony, fatty oils, storax, copaiba (rarely met with now, however) and the so-called synthetic balsam of Peru.

The last named is a thick black liquid, closely resembling the natural balsam, made from synthetically prepared esters with various oleo-resinous matters in order to give it the proper consistency.

In addition to the Pharmacopœial tests, the balsam should be examined as follows:—

The acid and ester values should be determined. The acid value is usually from 70 to 80, and the ester value 180 to 200. A high acid value and low ester value indicates the presence of colophony or copaiba.

Solubility in alcohol. The balsam is practically entirely soluble in 90 per cent alcohol. Fatty oils will be detected by the insolubility of the sample. The iodine value varies from 38 to 45.

The refractive index at 20° varies between 1.585 and 1.595.

The copper acetate and petroleum ether test described under balsam of tolu is not altogether reliable. A faint green colour is often produced by pure balsam, but a brilliant emerald green is strongly indicative of the presence of colophony.

If the balsam be extracted with petroleum ether and the residue obtained by evaporation of the solvent be treated with a drop of HNO_3 , a marked green coloration is indicative of the presence of colophony.

BALSAM OF TOLU.

Balsam of Tolu is obtained by making incisions in the trunk of *Myroxylon toluifera*. It is an aromatic balsam, official in the British Pharmacopœia, which authority describes it as a soft, tenacious solid becoming harder on keeping and, in cold weather, brittle. Pressed between pieces of glass by the aid of heat, and examined with a lens, it exhibits an abundance of crystals. It is soluble in alcohol, the solution having an acid reaction. If 5 grms. are warmed with two successive portions of 25 c.c. and 10 c.c. of CS_2 , the solutions should yield, when evaporated, a distinctly crystalline residue which should require not less than one-third of its weight of KOH for its saponification (presence of a due proportion of benzoates and cinnamates). No other official tests are given.

Balsam of Tolu contains benzyl benzoate, benzyl cinnamate, cinnamic and benzoic acids, traces of vanillin and the benzoic and cinnamic esters of an alcohol, tolueresinotannol, $C_{16}H_{14}O_3OCH_3OH$.

The principal adulterant of balsam of Tolu is colophony, but other resinous matter is sometimes added, and occasionally balsam already exhausted by water. The examination of this drug should embrace, in addition to the Pharmacopœial tests, the determination of its solubility in various solvents, and the acid and ester values. The following are the minimum solubilities of a genuine balsam :—

In 90 per cent alcohol	Per cent 90
„ chloroform	95
„ petroleum ether	2 to 8

The acid value of the balsam varies from about 105 to 140 rarely up to 150; and the ester value from 35 to 70. In the presence of colophony or exhausted tolu balsam, the ester value is lowered.

The presence of colophony is confirmed by the following test: 5 grms. of the sample are exhausted with petroleum ether (preferably by first rubbing down the balsam with a little CS_2 to make it viscous), and the filtered petroleum solution is shaken with an equal volume of 0.1 per cent aqueous solution of copper acetate. Copper abietate is soluble in petroleum ether, and therefore if colophony be present, the petroleum solution will show a marked emerald green colour. In the author's experience, pure samples will never give more than the faintest green tint under these conditions, 2 per cent of colophony giving a marked green colour.

Tincture of Tolu.—This drug is official, being a solution of two ounces of balsam in sufficient 90 per cent alcohol to produce 20 fluid ounces. It should have the following characters :—

Specific gravity	0.862 to 0.870
Solid residue	8.5 „ 9 grms. per 100 c.c.
Alcohol by volume	81 „ 84 „

BENZOIN.

Although there are many varieties of benzoin or gum Benjamin, as it is often termed, found in commerce, the official drug in the British Pharmacopœia is restricted to the products known as Siam and Sumatra benzoin. This is probably not the intention, however, of the compilers, since the words “and probably from other species of *styrax*” are used, which would appear to allow the use of Penang and other benzoin. The only tests given in the British Pharmacopœia are that the drug is to be almost entirely soluble in 90 per cent alcohol and in solution of potassium hydroxide.

The British Pharmaceutical Codex states that the ash should not exceed 2 per cent, nor the matter insoluble in 90 per cent alcohol more than 10 per cent.

Benzoin occurs in commerce in tears, lumps or blocks, according to quality and always contain some bark and mechanical impurities.

As this drug is used for the manufacture of "natural" benzoic acid, the proportion of that acid present is a matter of importance. For the manufacture of the tinctures (compound and simple) the percentage of matter soluble in 90 per cent alcohol is the principal consideration.

Ash.—The ash will naturally rise with the amount of woody fibre, bark, etc., present in the resin. Samples containing more than 2 or at most 2·5 per cent of mineral matter must be regarded as of inferior quality, and although no standard for this exists officially, this figure must be taken into account in judging the quality of samples.

Solubility in Alcohol.—Not less than 90 per cent should be soluble in 90 per cent alcohol. Solubility tests with other solvents afford no useful information.

Acid and Ester Values.—These figures should be determined, as the presence of other resinous matter is indicated by any wide variations from the following limits, which represent the analyses of 12 samples of benzoin of each type named, obtained from reliable sources, and having the ash and solubility values above mentioned :—

	Siam Benzoin.		Sumatra Benzoin.		Other Varieties.	
Ash	0·24 to 1·77 p.c.		0·4 to 1·82 p.c.		0·4 to 2·23 p.c.	
Soluble in 90 per cent alcohol . . .	90	96	91	93·5	90·5	93·9
Acid value	130	158	98	133	106	135
Ester value	42	68	58	98	60	89

Siam benzoin (made into a tincture and diluted with water) has a characteristic odour of vanilla, Sumatra benzoin rather recalls that of a mixture of styrax and vanilla, whilst Penang has an odour allied to that of styrax.

Estimation of Benzoic Acid.—Benzoin contains from 12 per cent to 20 per cent of benzoic acid, sometimes as much as 22 per cent. It may be approximately determined by powdering the sample, mixing it with twice its weight of sand, and heating it in a beaker covered with a perforated filter paper. The benzoic acid sublimes and may be condensed in a porcelain or other cone kept well over the top of the beaker, and kept as cool as possible by any suitable means.

Tincture of Benzoin (compound).—This tincture, known also as friar's balsam, is prepared with 90 per cent alcohol, by extracting benzoin, storax, balsam of tolu and socotrine aloes.

It is obvious that such a complex mixture allows of no separation of its ingredients that will give any approximate quantitative results.

The specific gravity of properly prepared compound tincture of benzoin varies between 0·890 and 0·904. It should contain 75 per cent of alcohol, and not less than 18 grms. of solid resinous matter per

100 c.c. This is not a standard given by the British Pharmacopœia, but is based on the fair average values of the soluble matter in the drugs employed for the manufacture of the tincture. Samples containing 17 per cent of residue would probably not be taken exception to, but a tincture yielding anything below this must be regarded as having been carelessly prepared, or made from drugs containing too little soluble matter.

From the description of benzoin in the Pharmacopœia, it should certainly not contain under 90 per cent of soluble matter. Purified styrax never contains less than 90 per cent of soluble matter; the same is true for balsam of tolu, and socotrine aloes contains from 80 per cent to 86 per cent of matter soluble in 90 per cent alcohol. The use of low-grade benzoin containing 60 per cent to 75 per cent of soluble matter, and much mechanical impurities, or of crude storax, containing less than 60 per cent of soluble matter is often the cause of the production of an inferior tincture. It is to be remembered that a certain amount of volatile solid matter is lost when drying the tincture, but this is allowed for by the above comparative figures which were all obtained by heating the extract of the drug to constant weight. Dowzard ("Chemist and Druggist," 20 Feb. 1904) recommends drying with about 10 per cent of the weight of the tincture of freshly ignited magnesium oxide. This fixes some of the volatile matter, and gives a result about 2 per cent higher than when dried without such addition.

CANNABIS INDICA.

Cannabis Indica, the dried flowering or fruiting tops of the female plant of *Cannabis sativa*, is official in the Pharmacopœia. It is used for the preparation of a tincture and an extract, both of which are official. No official standards are given.

This drug, which is known as guaza (Bombay variety) or ganjah (Bengal variety) is closely related to "bhang" or "hashish" of the native Indians. This latter is the dried leaf of the plant, whilst the "charas" or "churrus" is the resin extracted by heating the plants in a cloth.

The narcotic effect of this drug is produced by a resinous substance known as cannabinone. The principal constituent of this oleo-resinous matter is cannabinol, a dark-coloured oil of the formula $C_{18}H_{24}O_2$, boiling at 265° and of specific gravity 1.0424. Traces of a levorotatory terpene (probably an olefinic terpene) and a sesquiterpene (cannabinene) are also present, and a minute quantity of the alkaloid choline.

Cannabis indica yields from 14 per cent to 17 per cent of mineral matter.

The following table represents the (1) amount of extract with 90 per cent alcohol, (2) the same washed with water, (3) ether-alcohol-soluble resins, (4) per cent of (1) soluble in water, (5) moisture, in a number of type samples examined by David Hooper:—

	Rectified Spirit Extract	Washed Spirit Extract.	Ether- and Spirit- Soluble Resins.	Per cent of Spirit Extract Soluble in Water.	Moisture in Ganjah.
	Per cent	Per cent	Per cent	Per cent	Per cent
Bengal, Navagon 1	23.6	21.2	21.8	10.1	9.0
" " 2	22.1	20.4	20.1	7.6	7.1
" " 3	21.1	19.5	18.8	7.6	6.7
" " 4	19.8	18.1	18.4	8.5	9.2
Bombay, Sholapur (exported)	20.9	19.4	20.1	7.1	7.1
" Khandesh	18.0	16.5	16.8	8.3	7.4
" Satara	17.8	16.6	16.6	7.0	8.9
Independent State, Hydera- bad	17.7	16.8	16.5	5.0	7.6
N.W. Provinces, Basti . .	17.2	15.8	—	8.1	10.3
Central Provinces, Nimar . 1	16.7	15.0	15.6	10.2	8.4
" " " " 2	15.1	13.7	14.4	9.2	8.6
Bombay, Ahmednagar . . 1	16.7	15.4	15.4	7.7	12.4
" " 2	16.2	15.2	14.9	6.1	11.6
" " " " 3	14.6	13.4	13.3	8.2	9.8
" Nasik	16.8	14.3	14.3	14.8	8.2
" Sholapur	14.8	14.0	13.9	5.4	8.4
N.W. Provinces, Ghazipur .	17.1	13.7	13.8	19.8	10.0
Sind	16.3	13.9	14.7	14.7	8.4
Bombay, Surat	15.6	13.4	14.1	14.3	10.0
" Bijapur	14.5	13.4	13.4	7.5	9.2
Madras, Kistna Dist. . . .	31.0	24.0	23.4	22.5	7.6
" Ootacamund	28.1	20.8	20.1	25.9	9.8
" Ganjam	23.7	18.0	17.6	24.0	10.3
" Bangalore	21.6	17.0	17.3	21.3	8.2
" Tanjore	24.1	15.9	16.1	34.0	9.7
" Madras City	19.4	13.0	13.2	32.9	7.9

In the above table the second column represents the amount in the first column washed with hot water, and then dried and weighed. The fourth column expresses the per cent of the amount in column No. 1 dissolved by the water. The third column is the amount of resin directly extracted from the air-dried drug with ether, and then extracting the alcoholic extract with ether, leaving the resin soluble in alcohol but not in ether; this residue is added to the direct ether extract and should correspond closely with the washed alcohol extract (column No. 2).

Extract of Cannabis Indica is the 90 per cent alcohol extract dried to the consistence of a soft extract. Well-made extracts have the following average characters:—

	Per cent
Water	4 to 8
Ash	1.5 „ 3.5
Water-soluble extract	6 „ 15
Soluble in 90 per cent alcohol	practically complete

Tincture of Cannabis Indica should have a specific gravity of 0.845 to 0.850; a solid residue of 3.5 grms. to 4.2 grms. of solid matters per

100 c.c. ; and should contain 85 per cent to 87 per cent of alcohol by volume.

CATECHU.

The official variety of this drug is the light-coloured extract of the leaves and grey shoots of *Uncaria gambier*. It occurs in commerce under the name of gambier, in small cubes about two-thirds of an inch in measurement each way. The official standards are that when examined under the microscope it will be found to consist chiefly of minute acicular crystals. It is almost entirely soluble in boiling water. At least 70 per cent should be soluble in 90 per cent alcohol. No reaction should be given for starch, and it should not yield more than 5 per cent of ash.

(Black catechu or cutch is an extract from the heartwood of *Acacia catechu*.)

Catechu consists of 10 to 30 per cent of a body called catechin, which is probably a phloroglucide of tetrahydro-protocatechuic acid, and 30 to 50 per cent of catechu-tannic acid.

Commercial catechu should contain from 8 to 10 per cent of moisture and from 3 to 5 per cent of mineral matter. Genuine samples should answer the requirements of the Pharmacopœia as given above, and should yield from 30 to 50 per cent of tannic acid when determined by Lowenthal's permanganate process (see p. 11). At least 45 per cent should be soluble in ether.

Catechu is sometimes adulterated with starch, which is detected by the iodine reaction with an aqueous decoction of the sample, and by a microscopic examination.

Chalk and calcium sulphate are common adulterants, up to 30 per cent being found in some samples. These are found in the usual manner in the ash.

The difference between gambier and cutch is indicated by a fluorescence test as suggested by Dieterich ("Pharm. Central. H." 1896, 855). Three grms. of catechu are dissolved in 25 c.c. of normal caustic alkali and 100 c.c. of water. Fifty c.c. of petroleum ether are added and the mixture well shaken. With pale catechu or gambier, the petroleum shows a green fluorescence, but with *acacia* cutch no fluorescence is shown.

Tincture of Catechu.—The characters of this tincture are given in the table on page 495.

CARDAMOMS.

The seeds of *Elettaria cardamomum* are official under the name of cardamom seeds. The Pharmacopœia describes the pericarps of the fruits and states that the seeds shall be kept in them until they are required for use.

A description of the fruits is given, but the only standard is that the seeds should not yield more than 4 per cent of ash.

According to the "Chemist and Druggist," (Diary, 1899, 500) the principal varieties imported are the following :—

Mysores.—Divided into rounds and longs. The former are what the B.P. calls "ovoid"; they vary in length from a quarter of an inch to four-fifths of an inch (the latter 1 in 10), and have a smooth pericarp of a cream colour, due to the use of bleaching agents. Their quality is judged by their weight. Sometimes the seeds are shrivelled (unripe), so that the fruit is husky. This is not so frequent in the longs, which are simply thinner than the rounds, and are not so smooth on the surface, nor so pale, as a rule. The B.P. description, "longitudinally striated," might exclude most of the rounds, as they look smooth until closely examined.

Malabars.—These are smaller than Mysores, and there is a greater proportion of seed to pericarp in them. They are fat pods, with a pointed apex. Generally pale-brown or pink and longitudinally striated. Rarely more than half an inch long. They have a full flavour.

Mangalores.—These are almost globular in shape and not unlike Malabars. All three are washed or bleached before exportation.

In addition to these there are the so-called "Ceylon Wilds" which are probably derived from another species.

It is clear that Mysore cardamoms are those usually employed, but the Pharmacopœial description would certainly allow the use of Malabar cardamoms also.

The only methods of examination of cardamom fruits or seeds are the determination of the ash value, a microscopic examination, and the estimation of the essential oil.

The ash of cardamoms is usually, in the case of Mysore seeds, well within the official requirements, but in the case of Malabar seeds, it is often as high as 8 per cent to 9 per cent, so that in such cases Malabar seeds would be excluded. According to Cowley and Catford, the following figures are average ones for the three varieties mentioned :—

Variety.	Malabar.	Mysore.	Mangalore.
Number of fruits in 10 grms. . .	80	55	45
Percentage proportion of pericarp . .	30	25	20
Percentage proportion of seed . .	70 { dark, 57 light, 13	75	80
Percentage of ash from dark seed . .	5·0	3·3	2·9
Percentage of ash from light seed . .	8·5 to 9	4·5	—
Percentage of ash from pericarp . .	13·0	7·1	7·6

Greenish ("Pharm. Journ." 4, XII. 264-393) is of opinion that the minimum limit for ash for the seeds should be 5·5 per cent, and states that the ash of the pericarp is so near this figure that the ash limit will not discriminate between the powdered seeds and the powdered whole fruits.

In the author's experience this is hardly the case, and the limits for ash for the seeds should be 6 per cent as a maximum, the pericarps yielding as much as 8.5 to 10.5 per cent.

Cardamom seeds should yield at least 3 per cent usually up to 4.3 per cent of essential oil when steam-distilled and the separated oil measured.

The characters of the essential oils yielded by various cardamoms are a matter of some uncertainty, but the author has examined the subject to some extent, and the following is the outcome of the examination of samples of reliable origin. A good deal of genuine oil distilled from good cardamoms, however, has an optical rotation of about $+30^{\circ}$.

—	Sp. gr. at 15.5° .	Optical Rotation at 16° (100 mm. tube.)
Oil of Malabar cardamoms . . .	0.9418	$+40^{\circ} 41'$
Oil of Mysore cardamoms . . .	0.9418	$+46^{\circ} 39'$

These figures are in fair agreement with those given for Malabar oil, but in no way resemble those quoted by Schimmel for Ceylon oil.

The oils were soluble with a slight opacity in 40 to 45 volumes of 60 per cent alcohol.

There is little difference between the two oils. On distillation at ordinary pressure, the oil, which is very rich in esters, in both cases decomposes partially, and a considerable quantity of free acid distills over. According to Weber ("Annalen," 238, 89), formic and acetic acids are found in the distillate. Acetic acid is undoubtedly the chief acid constituent of the esters, but the presence of formic acid could not be confirmed. If it is present, it is only in faint traces. On distillation under reduced pressure the earlier fractions (the boiling-point rises gradually until 50 per cent has distilled over) contain cineol, but only to the extent of 5 to 10 per cent of the oil. This figure is the result of an approximate estimation by means of phosphoric acid. The earlier fractions also contain one or more terpenes, amongst which is limonene. Weber states that terpinine is also present, but this is doubtful, nor could Schimmel find it in Malabar oil; and as it easily forms a well-defined nitrite when present, it cannot exist in an appreciable quantity. A small quantity of terpineol is present in both oils, and is easily identified by its phenyl-urethane. The terpineol comes over with the fraction obtained at 160° to 170° C. at 18 mm. The nature of the alcoholic constituent of the greater part of the esters requires further elucidation.

The following description is sufficient for the recognition of the pericarp in the powdered drug, as is recommended by Greenish to be included in the next edition of the Pharmacopœia:—

"Powdered cardamoms, when examined under the microscope,

should exhibit masses of thin-walled parenchymatous cells packed with minute starch grains; long straight epidermal cells with moderately thick walls, and small polygonal reddish-brown cells with very thick walls. It should be free from sclerenchymatous fibres or elongated cells, or small cells containing brown resin."

Compound Tincture of Cardamoms.—The characters of this official tincture are given in the table on page 495.

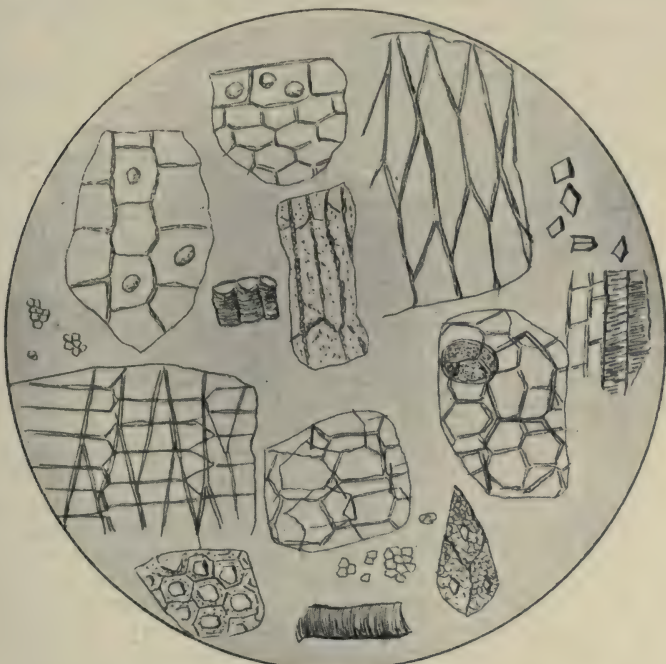


FIG. 40.—Powdered Cardamoms.

It is also to be noted that this tincture is optically active, on account of the sugar present which is derived from the raisins used in its preparation. A genuine sample should always be lævorotatory to the extent of $-2^{\circ} 10'$ to $-2^{\circ} 40'$, when cleared as described under sugars and examined in a 100 mm. tube. Dextrorotatory samples are always prepared with cane sugar to save the use of raisins, and should be condemned.

COPAIBA.

Copaiba, or balsam of copaiba (or capivi) is described in the British Pharmacopœia as an oleo-resin obtained from various species of *Copaifera*.

It is a mixture of resins and an essential oil, the Pharmacopœial standards being as follows: Specific gravity 0.916 to 0.993. It should

yield at least 40 per cent of essential oil leaving a hard friable residue. The essential oil is described as rotating the plane of polarization from -28° to -34° (100 mm. tube). The essential oil should not boil under 250° C. If 2 drops are dissolved in 20 parts of CS_2 and a drop of a cooled mixture of equal parts of nitric and sulphuric acid added, a transient violet colour is not produced (absence of gurjun balsam). If 4 drops be added to a mixture of half an ounce of glacial acetic acid and 4 drops of nitric acid, a reddish or purple-red colour should not result (absence of gurjun balsam).

This monograph is totally inadequate, and has been to some extent corrected by that in the Pharmaceutical Codex. Before discussing this important drug in detail it is necessary to call attention to the fact that the optical rotation of the British Pharmacopœia, besides being erroneous in itself, is for a tube of 200 mm., which fact was inadvertently omitted by the compiler of the monograph. The rotation limits thus corrected are far too narrow, and should read from -7° to -35° , or thereabouts. Further, the test with nitric and acetic acids, will not reveal the presence of gurjun balsam unless it be present to a fairly large extent: whereas if the test be applied to the essential oil instead of the balsam, it will be found to be very delicate. Oil of copaiba is also official, its specific gravity being given in the Pharmacopœia as 0.900 to 0.910.

The British Pharmaceutical Codex states that the acid value of the thick balsams varies from 77 to 83 and the ester number does not exceed 10. These figures are a little too stringent.

There are numerous varieties of copaiba found in commerce, the principal of which are Maranhão, Maracaibo, Cartagena, Bahia and Para balsams. In addition to these the following are sometimes met with: Surinam, Angostura, Maturin and British Guiana balsams.

Copaiba has for many years been subject to gross adulteration. Fatty oils and turpentine were at one time met with, but in the course of the last ten years the author has examined several hundred samples and has not met with any adulteration other than with either gurjun balsam or the so-called African copaiba, or frequently, a mixture of the two. For analytical purposes, copaiba is to be regarded as a mixture of an essential oil and a resin, which must be separated and the characters of the two examined. It is obvious that, since the essential oil is practically a mixture of neutral sesquiterpenes, such important characters as the acid and ester values will vary according to the percentage of essential oil, whilst they may be fairly constant for the resins present.

The thicker balsams, such as Maranhão, Maracaibo, etc., are those usually preferred for use in medicine, but the thinner ones, such as the Para and Bahia varieties, are used for the distillation of the essential oil.

In the examination of this drug, the following are the figures that should be obtained and the methods adopted. As the various balsams have somewhat different characters, the limit figures for each are summarized in tables (p. 449).

(1) The specific gravity of the balsam, and of the essential oil.

BALSAMS OF COPAIBA OF VARIOUS TYPES.

	Maranham.	Maracaibo.	Cartagena.	Bahia	Angostura.	Para.	Surinam.
Sp. gravity at 15° . . .	0.980 to 0.990	0.972 to 0.995	0.956 to 0.972	0.958 to 0.968	0.985 to 0.990	0.920 to 0.980	0.940 to 0.965
Refractive index at 20° . . .	1.508 " 1.512	1.504 " 1.512	1.508 " 1.512	1.504 " 1.509	1.510 " 1.516	1.504 " 1.516	1.502 " 1.512
Percentage of essential oil . . .	40 " 44	38 " 45	42 " 52	48 " 54	38 " 45	44 " 78	40 " 75
Acid value . . .	78 " 83	75 " 90	82 " 90	57 " 60	78 " 88	30 " 65	25 " 60
Ester value . . .	10 " 18	5 " 18	5 " 15	10 " 12	10 " 16	5 " 30	10 " 20
Optical rotation . . .	—	—	—	Practically inactive.	+ 25°	- 20° " - 40°	—
RESINS FROM THE ABOVE BALSAMS.							
Acid value . . .	125 to 135	120 to 140	130 to 140	115 to 130	120 to 135	110 to 128	90 to 129
Ester value . . .	30 " 35	15 " 35	15 " 35	20 " 35	20 " 40	10 " 20	15 " 30
ESSENTIAL OILS FROM THE ABOVE BALSAMS.							
Sp. gravity at 15° . . .	0.900 to 0.904	0.897 to 0.904	0.896 to 0.905	0.905	0.904	0.910 to 0.905	0.910
Refractive index at 20° . . .	1.5050	1.5075	1.5060	1.5065	1.5065	1.5075	1.5000
Boiling-point . . .	about 250°	about 250°	about 250°	about 250°	about 250°	about 250°	about 250°
Optical rotation . . .	about - 10°	- 6 to - 12°	- 6° to - 16°	variable.	- 2° to - 12°	up to - 70°	—

Whilst the former varies within very wide limits, the latter will be found to be much more constant.

(2) The refractive index of the balsam and the oil.

(3) The optical rotation of the essential oil.

(4) The amount of essential oil and resin. This may be determined by drying about 2 grms. in a flat platinum capsule at about 120° C. in an air oven to constant weight. The essential oil, however, should be separated for examination by passing a brisk current of steam through 30 grms. of the balsam. This will yield sufficient oil for examination, and if the distilling flask be kept at 100° by immersion in a water bath whilst the steam is passing briskly through, the distillation will be complete in from three to four hours. If the oil be distilled over a naked flame, it should be under reduced pressure, but even then it is not possible to decide when all the oil has passed over, and some decomposition is bound to occur, with the result that the essential oil is contaminated with products of destructive distillation of the resin.

(5) The following is the only reliable colour reaction that can be used. It will detect very small additions of gurgun balsam. Five or six drops of the essential oil are added to a mixture of 15 c.c. of glacial acetic acid, and 5 drops of nitric acid. If no coloration takes place in five minutes, gurgun oil may be regarded as absent. In the presence of this adulterant a red or purple-red coloration is developed in a minute or two, the time taken and depth of colour depending on the amount of adulteration.

(6) The acid and ester values of the balsam and of the separated resin should be determined. The residue left in the distilling flask should be cooled, pressed between filter paper to remove as much moisture as possible, and then dried in a water oven.

The figures on page 449 represent fair average values, but it must not be forgotten that from time to time abnormal samples will be found which have figures outside these limits, but are still pure.

The necessity of insisting on a high optical rotation for the essential oil is shown by the following table which includes the values determined on a number of samples of direct importation by Messrs. Evans, Sons, Lescher & Webb's chemists:—

MARANHAM.

Specific Gravity.	Optical Rotation.	Specific Gravity.	Optical Rotation.
0.902	- 13° 0'	0.899	- 16° 12'
0.900	- 20° 0'	0.898	- 17° 30'
0.900	- 16° 0'	0.898	- 10° 0'
0.898	- 17° 20'	0.905	- 9° 30'
0.900	- 21° 40'	0.900	- 13° 20'
0.901	- 14° 10'	0.902	- 16° 40'
0.902	- 13° 0'	0.903	- 16° 12'
0.900	- 13° 44'	0.0025	- 12° 30'
0.904	- 10° 30'	0.899	- 17°
0.901	- 15° 0'	0.898	- 18°

MARACAIBO.

Specific Gravity.	Optical Rotation.	Specific Gravity.	Optical Rotation.
0.903	- 6° 30'	0.900	- 6° 0'
0.900	- 7° 0'	0.902	- 6° 0'
0.901	- 6° 0'	0.901	- 8° 0'
0.898	- 21° 0'	0.895	- 19° 30'
0.890	- 30° 0'	0.895	- 20° 36'
0.894	- 21° 30'	0.887	- 26° 26'
0.888	- 28° 0'	0.896	- 18° 30'
0.896	- 20° 40'	0.893	- 28° 0'
0.894	- 26° 0'	0.886	- 32° 40'
0.891	- 28° 30'	0.891	- 28° 0'
0.893	- 23° 44'	0.892	- 25° 0'
0.891	- 24° 0'	0.886	- 31° 0'
0.889	- 26° 0'	0.889	- 26° 0'
0.886	- 31° 0'		

CARTAGENA.

Specific Gravity.	Optical Rotation.	Specific Gravity.	Optical Rotation.
0.896	- 30° 0'	0.895	- 40° 0'

BAHIA.

Specific Gravity.	Optical Rotation.	Specific Gravity.	Optical Rotation.
0.898	- 9°	0.898	- 8°
0.897	- 10°	0.909	- 2° 42'
0.888	- 28°	0.901	- 8° 0'

Adulterants.—As stated above, the only adulterants met with to any extent at the present time are African copaiba and gurgun balsam. The colour reaction described above (p. 450) will detect as little as 5 per cent of gurgun oil with certainty. Dextrorotatory oils, or oils with a rotation below -6° are very suspicious and African balsam of copaiba is to be suspected. Under oil of copaiba the British Pharmacopœia states that the oil is soluble in its own volume of absolute alcohol and gives this as a distinction from African copaiba oil. This, however, is not so, as both are usually soluble in their own volume of absolute alcohol.

The following characters of these two adulterants will assist the analyst in forming an opinion on the character of the sample examined.

	Gurjun Balsam.	African Copaiba.
Specific gravity at 15°	0.955 to 0.980	0.985 to 1.000
Specific gravity of essential oil	0.910 „ 0.930	0.916 „ 0.925
Rotation of essential oil	up „ - 135°	+ 12° „ + 45°
Refractive index of essential oil	1.5050	1.5000 „ 1.5080
Acid value of balsam	10 „ 20	55 „ 60
Ester value of balsam	1 „ 12	10
Acid value of resin	40 „ 80	110 to 120
Ester value of resin	2 „ 25	20

In cases where African copaiba is suspected, the essential oil may often be fractionated under reduced pressure, with advantage. African copaiba oil yields fractions becoming steadily more dextrorotatory—so that if an oil from a given sample shows a rotation of say -4° and on fractionation, the fractions become less levorotatory and then dextrorotatory, it is almost certain that African copaiba is present. The following figures were obtained on three authentic samples of African copaiba by the author and Bennett:—

Fraction.	I.			II.			III.		
	Sp. Gravity.	Ref. Index.	Rotation.	Sp. Gravity.	Ref. Index.	Rotation.	Sp. Gravity.	Ref. Index.	Rotation.
25 per cent	0.917	1.5030	+17°30'	0.915	1.4960	+16°	0.914	1.4975	+24°
25 „	0.918	1.5043	+28°30'	0.917	1.4965	+19°	0.917	1.4980	+26°
25 „	0.921	1.5061	+46°	0.920	1.4980	+24°	0.919	1.4981	+29°
20 „	0.927	1.5082	+55°	0.924	1.5089	+48°	0.923	1.5090	+43°

These results have been confirmed by Cocking ("Chemist and Druggist," 1910, II., 51) who gives the following table showing the optical value of pure and adulterated copaiba oils, and of their 10 fractions of 10 per cent each. He points out that if the sample be pure the figures obtained will all be negative, and they will increase from the first to the last fraction, although not regularly. If the rotation of the first fraction be subtracted from that of the tenth, a figure will be obtained which varies very little for genuine samples, and is always a negative quantity. This figure (the "difference value") varies from -3.7° to -7.6° .

When African copaiba is examined in this manner, the rotations of all the fractions are, as would be expected, dextrogyrate, and the rotations of the successive fractions increase, but to a much greater extent than with the South American copaiba, in consequence of which the difference value is much greater than with copaiba and is a positive figure. The figures also show a curious feature in that the tenth fraction has a considerably lower rotation than the ninth. As would be expected from the fact that the range of boiling-points of the

constituents of the volatile oils from the two varieties are practically identical, a mixture of the two will distil over containing proportional parts in each fraction, and the presence of the African will be shown at once by the difference value being positive.

In some cases, as will be seen from the tabulated results below, where only a small percentage of the adulterant was present, all the fractions were lævogyrate, but the difference value was positive. The same process was applied to gurjun oil, which, like copaiba, gives lævogyrate fractions, but, unlike it, they successively decrease instead of increasing, and thus give a positive difference value similar to African copaiba.

With the true copaibas the rotation of the first fraction is in every case lower than that of the original oil, but in the adulterated samples it is higher. It is important that the distillation of the oil should be conducted *in vacuo*, since, if carried on under atmospheric pressure, the higher temperature necessary causes some decomposition, which entirely alters the optical rotation, as shown in the table on page 454 under "u".

The presence of such adulterants as fatty oils or turpentine gives no difficulty to the analyst. In the case of fatty oils the sample is saponified and the liquid neutralized, the acids precipitated with silver nitrate and the mixture diluted with water. The salts of the resin can be shaken out with ether, in which the fatty acid salts are insoluble. These can be decomposed by hydrochloric acid and the liberated fatty acid examined. Their liquid or semi-liquid character enables them to be at once distinguished from the resin acids.

Turpentine is at once detected by its odour on evaporation. Its boiling-point—about 160°—enables it to be easily distilled off before the essential oil of copaiba passes over, and its refractive index, about 1.4720, at once discriminates it from oil of copaiba.

CREOSOTE.

This drug is obtained by the distillation of wood tar. It is official in the British Pharmacopœia which describes it as a mixture of guaiacol, creosol and other phenols. It requires it to have the following characters:—

It is to be neutral or only slightly acid to litmus; soluble in 150 volumes of water at ordinary temperatures; specific gravity not below 1.079; it is soluble in alcohol, ether, chloroform, glycerin, and glacial acetic acid. It distils between 200° and 220°. A 1 per cent alcoholic solution gives a green coloration, rapidly changing to reddish-brown, with a drop of ferric chloride solution. It is lævogyrotatory. A drop on filter paper, heated to 100°, leaves no translucent stain. It is miscible with an equal volume of collodion without gelatinization; when shaken with five times its volume of ammonia (sp. gr. 0.959), its volume is not materially diminished (distinction from phenol).

As a matter of fact, beechwood creosote is optically inactive, or faintly dextrorotatory.

OPTICAL ROTATIONS OF THE VOLATILE OILS AND FRACTIONS THEREOF.

I.—*South American Copaibas.*

—	Original Oil.	First Fraction.	Second Fraction.	Third Fraction.	Fourth Fraction.	Fifth Fraction.	Sixth Fraction.	Seventh Fraction.	Eighth Fraction.	Ninth Fraction.	Tenth Fraction.	Difference Value.
<i>a</i>	Deg. -19.8	Deg. -17.3	Deg. -17.6	Deg. -18.1	Deg. -18.0	Deg. -18.4	Deg. -19.1	Deg. -19.9	Deg. -20.7	Deg. -21.8	Deg. -22.8	Deg. -5.5
<i>b</i>	-15.2	-14.1	-14.6	-15.3	-15.4	-16.0	-16.0	-16.9	-17.7	-19.0	-21.7	-7.6
<i>c</i>	-19.9	-17.4	-17.9	-18.4	-18.7	-19.2	-19.5	-20.1	-21.0	-22.0	-23.8	-6.4
<i>d</i>	-19.4	-17.8	-18.3	-18.4	-18.5	-18.6	-18.8	-18.8	-19.6	-20.1	-23.0	-5.2
<i>e</i>	-22.0	-19.8	-20.6	-20.8	-21.1	-21.3	-21.4	-21.9	-22.8	-24.0	-25.7	-5.9
<i>f</i>	-15.7	-13.6	-14.4	-14.4	-14.7	-15.0	-15.0	-15.1	-15.7	-16.6	-19.0	-5.4
<i>g</i>	-22.9	-21.3	-21.3	-21.1	-21.5	-21.7	-22.8	-23.0	-23.8	-24.5	-27.9	-6.6
<i>h</i>	-13.2	-12.4	-12.6	-13.0	-13.2	-13.8	-14.0	-14.6	-15.5	-16.1	-17.8	-5.4
<i>i</i>	-19.9	-18.0	-18.7	-18.3	-19.0	-19.0	-19.2	-19.6	-20.2	-20.8	-23.2	-4.2
<i>j</i>	-22.9	-20.6	-20.4	-20.5	-20.8	-21.0	-22.0	-22.9	-23.4	-25.7	-27.9	-7.3
<i>k</i>	-18.8	-17.6	-18.5	-18.5	-18.8	-18.8	-18.8	-19.4	-20.0	-20.8	-22.5	-4.9
<i>l</i>	-21.1	-19.4	-20.4	-20.2	-20.7	-20.9	-21.2	-21.6	-21.9	-22.3	-23.1	-3.7
II.— <i>African Copiba, Gurjun Balsam, and Adulterated Copibas.</i>												
<i>m</i>	+29.7	+14.4	+18.5	+18.9	+29.7	+33.1	+37.9	+45.3	+50.8	+57.8	+37.5	+23.1
<i>n</i>	+33.7	+19.0	+23.5	+26.4	+30.6	+33.4	+36.3	+39.8	+45.3	+53.6	+41.4	+23.4
<i>o</i>	+1.8	-4.8	-4.4	-4.6	-8.2	-0.1	+6.4	+10.2	+14	+4.1	+3.0	+7.3
<i>p</i>	-3.9	-5.5	-5.5	-4.9	-4.9	-4.7	-4.0	-3.9	-3.0	-2.7	-1.8	+3.7
<i>q</i>	-7.6	-8.4	-8.3	-7.9	-7.9	-7.6	-7.7	-7.7	-7.7	-7.2	-7.2	+1.2
<i>r</i>	-4.5	-6.3	-6.2	-6.3	-6.4	-5.7	-5.1	-4.3	-4.0	-3.3	-0.9	+5.4
<i>s</i>	-93.2	-109.4	-107.8	-111.4	-105.4	-100.6	-95.6	-96.2	-90.6	-80.6	-65.0	+44.4
<i>t</i>	-7.0	-7.3	-6.9	-6.8	-6.8	-6.9	-6.9	-6.9	-6.6	-6.5	-6.2	+1.1
<i>u</i>	-22.9	-9.3	-10.0	-10.2	-10.4	-10.5	-11.0	-11.5	-11.4	-9.6	-10.4	-1.1

a to *j*, Maranham. *k* and *l*, Pará. *m* and *n*, African. *o* and *p*, Maracaibo containing African. *g*, Central American containing African. *r*, Mixture composed of African ($\frac{1}{2}$) 25 per cent. and Maranham ($\frac{1}{2}$) 75 per cent. *s*, Gurjun balsam. *t*, Supposed to be Pará, but gave strong colour-reaction for Gurjun balsam. *u*, Maranham, same oil as *g*, but distilled under atmospheric pressure.

Creosote is usually distilled from beechwood—sometimes from oak or pine. It consists essentially of a mixture of phenoloid compounds in varying proportion, amongst which are phenol (boiling at 182°); paracresol (203°); guaiacol (200°); creosol (219°); dimethyl-guaiacol (230°); and propyl-guaiacol (241°).

Guaiacol is present to the extent of about 15 per cent to 25 per cent and is one of the most important constituents of creosote.

A good creosote should have a specific gravity of at least that required by the British Pharmacopœia, preferably a little higher—up to 1.085. On fractionation three typical samples gave the following results, with which pure samples will approximately correspond:—

	Sp. Gr.	Guaiacol.	Under 200° .	200° - 205° .	205° - 210° .	210° - 215° .	215° - 220° .
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	1.0815	21.5	6	39	22	25	6
2	1.0820	19.8	7.5	40	20	23	7
3	1.0800	23	5	35	24	22	10

In order to differentiate between creosote and coal-tar phenols (coal-tar creosote), one volume of the sample is shaken with a mixture of 3 volumes of glycerine and 1 of water. The diminution in the volume of the creosote indicates the amount of soluble impurities derived from the coal-tar.

A pure wood creosote loses at least 10 per cent of its volume when shaken with 5 volumes of 10 per cent ammonia.

Guaiacol is usually present to the extent of 10 per cent to 25 per cent, and may be determined as follows (Kebler, "American Jour. Pharm." 39, 933). Five c.c. of creosote are mixed with 50 c.c. of a 20 per cent alcoholic solution of potassium hydrate. The crystalline mass, which separates in 10 minutes to 30 minutes, consists of a compound of guaiacol and creosol with potassium. The dried crystals are heated for a moment with 5 c.c. of a 10 per cent solution of sulphuric acid, the liquid is diluted, and the mixture of guaiacol and creosol, which separates as a heavy oil, removed. By treating this oil with 4 c.c. of a concentrated solution of ammonia, the guaiacol ammonium compound is formed as a crystalline mass, which separates before the less crystalline creosol compound. The latter is removed by means of benzol, and the guaiacol ammonium compound decomposed by a 10 per cent solution of sulphuric acid. The liberated guaiacol is dissolved by shaking with benzol; and finally weighed after evaporating the solvent.

If a fuller analysis of creosote is required, Behal and Choay "Comptes Rendus" cxvi. 200) advise a separation based on the following facts:—

(1) Hydrobromic acid removes methyl from the methyl ethers of the phenols, (2) that monophenols can be removed by steam, (3) that

polyphenols cannot be thus removed, (4) that ether abstracts from aqueous solutions pyrocatechin and homopyrocatechin as well as monophenols, and (5) that pyrocatechin and homopyrocatechin are separable by benzene. In carrying out the process a current of hydrobromic acid is passed into creosote mixed with some water, by which proceeding the ethers of polyphenols are demethylated. By distillation with steam the monophenols are carried over and can be separated from the distillate by shaking with ether.

CUBEBS.

The dried full-grown unripe fruits of *Piper cubeba* are the official cubebs of the British Pharmacopœia.

The only official test is that the crushed fruit should impart a crimson colour to sulphuric acid.

The principal constituent of this drug is from 12 per cent to 15 per cent of essential oil (see p. 610). It also contains resinous matter, so that by extraction with ether it yields from 20 per cent to 22 per cent of oleo-resin. Cubebs yield from 6 per cent to 8 per cent of ash, usually about 7 per cent. Traces of an acid, termed cubebic acid, are responsible for the crimson colour with H_2SO_4 . This drug is frequently found in commerce mixed with similar fruits. The various piperaceous and other fruits used for adulterating cubebs may be, for the most part, distinguished from true cubebs simply by their external characteristics, whilst others resemble the genuine drug so closely that a microscopic examination of a section of the fruit is necessary. A few of the adulterants, however, can only be distinguished by the fact that they do not contain cubebic acid, and therefore do not give a purple-red coloration with strong sulphuric acid. Cubebic acid does not occur in the perisperm only, as has hitherto been supposed, but also in the pericarp, and the same is true of the occurrence of piperine in black pepper. Piperaceous plants which contain cubebic acid or an allied compound do not, as a rule, contain any alkaloid, such as piperine.

The characters of genuine cubebs are those given above, and as the percentage of essential oil is high, a determination of this should be made: 125 grms. well bruised, should be steam-distilled until no more oil is carried over. At least 12 per cent should be obtained from good cubebs, and this should have the characters described under oil of cubebs (p. 610). The crushed fruits should yield about 20 per cent or more of oleo-resin to ether, the ether being driven off at a temperature of 70° to 80°, and the residue weighed. The characters of tincture of cubebs will be found in the table on p. 495.

GALBANUM.

This gum resin is officially described as the product of *Ferula galbaniflua* and probably of other species.

The only characteristic test given in the Pharmacopœia is that if a fragment is heated to redness in a dry test tube, the contents of the tube, after cooling, yield with boiling water a solution which when

largely diluted with water and rendered alkaline with ammonia exhibits a blue fluorescence.

This test enables galbanum to be detected in certain other gum resins, such as ammoniacum.

The official galbanum is that known as Levant galbanum, and for medicinal purposes it must be in tears either separate or agglutinated. The lumps containing tears embedded in a resinous mass are therefore not official, but are frequently met with in commerce.

Galbanum contains about 5 per cent to 10 per cent of essential oil; in very soft varieties, as much as 20 per cent being found. It contains from 20 per cent to 30 per cent of gum and mechanical impurities and from 60 per cent to 70 per cent of resin soluble in alcohol. It also contains traces of free umbelliferone, which is the anhydride of umbellic acid $C_7H_6O \cdot COOH$; about 20 per cent of the resin consisting of umbelliferone (or umbellic acid) combined as an ester with the alcohol galbaniresinotannol $C_{18}H_{29}O_2 \cdot OH$. It is to the umbelliferone that galbanum owes the characteristic fluorescent reaction described above. A petroleum ether extract of galbanum should yield only a very slight green coloration when shaken with aqueous copper acetate. Pure galbanum should have the following characters:—

Mineral matter, 5 to 8 per cent	
Resin soluble in 95 per cent alcohol not below 55 per cent	
Acid value	20 to 40
Ester value	60 „ 100
Saponification value	80 „ 120

GUAIAACUM.

Guaiacum resin, the product of *Guaiacum officinale* or of *G. sanctum*, is official in the Pharmacopœia.

The characteristic official test is that an alcoholic solution assumes a blue colour on the addition of dilute ferric chloride solution.

Guaiacum usually occurs in large blocks, but sometimes in tears. It breaks with a clean glassy fracture, showing a greenish or reddish-brown colour.

The principal constituents of this resin are α -guaiaconic acid $C_{22}H_{24}O_6$; β -guaiaconic acid $C_{21}H_{26}O_5$; guaiaretic acid, $C_{20}H_{23}O_3(OH)$ (about 10 per cent); guaiacic acid $C_{21}H_{19}O_4(OH)_3$ (10 to 12 per cent); and small quantities of gum and other indefinite substances. This resin is of great interest on account of the fact that Doebner has succeeded in condensing tiglic aldehyde, guaiacol and cresol to a resinous acid, $C_{20}H_{24}O_4$, isomeric with guaiaretic acid, a result which throws some light on the formation of natural resins, and indicates that they owe their origin to the condensation of phenols and aldehydes rather than to the oxidation of the terpenes.

Good guaiacum resin in tears yields about 1 per cent of mineral matter and 98 per cent soluble in 90 per cent alcohol. Block guaiacum contains more mechanical impurities than the tears and usually gives 3 per cent of ash and about 90 per cent soluble in 90 per cent alcohol.

Numerous oxidizing agents produce a blue colour when brought

into contact with an alcoholic solution of guaiacum (this is due to the oxidation of *a*-guaiaconic acid).

The well-known reaction for blood with tincture of guaiacum is based on this fact.

Genuine guaiacum should have the following characters:—

	Per cent.
Mineral matter	1 to 4
Acid value	60 to 70
Soluble in 90 per cent alcohol	87 to 98
Acid value of acetylated resin	not above 50
Ester value of acetylated resin	125 to 150
Methoxyl number	70 to 85
Soluble in petroleum ether	not above 2

The ester value of the acetylated resin is valuable on account of the large amount of hydroxy-bodies present in this resin; and the high methoxyl number is characteristic.

Colophony as an adulterant of powdered guaiacum may be detected by the Storch-Morawski reaction (p. 478).

Starch is sometimes added and may be detected by testing the cold aqueous decoction with iodine solution.

Guaiacum adulterated with colophony will yield a large proportion of resin soluble in petroleum ether.

Ammoniated Tincture of Guaiacum.—This is official in the Pharmacopœia, but no standards are given. It is a solution of the resin in alcohol and ammonia, flavoured with essential oils of lemon and nutmeg. A pure tincture should have the following characters:—

Specific gravity	=	0.898	to	0.907	
Solid residue	=	14	„	17.5	per cent
Alcohol by volume	=	69	„	71	„
Ammonia NH ₃	=	1.9	„	2.2	„ (by weight)

The alcohol should be determined by rendering the tincture exactly neutral with dilute H₂SO₄ and then distilling the alcohol and determining the specific gravity of the distillate made up to the proper volume.

Ammonia is determined by distilling 25 c.c. rendered alkaline with KOH and diluted with 175 c.c. of water and collecting 100 c.c. of the distillate through a well-cooled condenser, and then titrating with decinormal H₂SO₄.

GAMBOGE.

Under the name Cambogia, this is official in the Pharmacopœia. It is a gum-resin obtained from *Garcinia Hanburii*.

The official standards are that it is completely dissolved by successive treatment with 90 per cent alcohol and water; that a cooled aqueous solution should not become distinctly green with solution of iodine (absence of more than a trace of starch); and that it should not yield more than 3 per cent of ash.

This drug consists of about 70 to 80 per cent of resin acids, known as "gambogic acid"; 15 per cent of gum; with small quantities of mineral matter, vegetable debris, etc.

It usually occurs in commerce in the form of pipes, the gum resin having been allowed to dry inside hollow bamboos. This type of gamboge is produced in Siam, Cochin China and Cambodia. The product of *Garcinia Morella*, a tree growing in India and Ceylon, is known as Indian gamboge. It is found in pieces of irregular shape, but should have the same characters as Siam gamboge. The usual adulterants are starch, rosin, turmeric and mineral matter. Starch is detected by its reaction with iodine, and also microscopically, mineral matter is detected by a high ash value, and rosin by the Storch-Marowski reaction (heating the powdered sample with acetic anhydride and allowing H_2SO_4 of specific gravity 1.5 to run slowly on to the surface of the cooled liquid when a violet colour is developed at the surface of contact of the liquids if rosin be present).

Rosin is also indicated by the high acid and low ester value of the sample. Ten samples of pure gamboge, examined by the author, gave the following figures:—

	Acid Value.	Ester Value.	Saponification Value.
1	76	60	136
2	81	58	139
3	82	64	146
4	74	60	134
5	73	60	133
6	81	55	136
7	84	61	145
8	80	69	149
9	73	62	135
10	78	51	129

Eberhardt gives the following limits for the reaction for starch:—

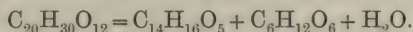
One gm. of the powder to be tested is dissolved in 5 c.c. of potash, followed by an addition of 45 c.c. of water, and finally an excess of hydrochloric acid. The turbid liquid is then filtered through cotton wool, and one or two drops of iodine are added to the clear filtrate. In presence of over 2 per cent of starch there immediately ensues a dark blue coloration, or a similarly coloured precipitate is formed. The powdered commercial drug usually gives a yellow coloration, which afterwards turns blue; pure gamboge, with 1 per cent of added starch, gives a dull blue, which deepens on standing, and deposits a precipitate after several hours. Five per cent to 10 per cent of starch gives a blue precipitate immediately. Five per cent and under of turmeric gives a decided starch reaction. Turmeric may also be detected by the borax reaction (see under Turmeric).

GENTIAN.

The dried rhizome and roots of *Gentiana lutea* are official in the Pharmacopœia, but no standards are given.

The fresh root contains at least three bitter principles, gentiopierin,

gentiin and gentiamarin, of which the last two exist in the dried drug the first having been decomposed by changes taking place during drying. Gentiopierin is a glucoside of the formula $C_{20}H_{30}O_{12}$ which yields gentiogenin and dextrin on hydrolysis, according to the equation



Amongst the other substances present are sugar, gentianose, which yields on hydrolysis gentiobiose and levulose, the former finally splitting up into dextrose.

The only methods of analysis available are the determination of the ash and the amount of extractive; and a microscopical examination.

Genuine gentian root, which is largely sold in powder, should not contain more than 5 per cent of mineral matter. The cold water extract of a good root varies from 30 per cent to 40 per cent (but in a highly fermented root this may be much lower). The amount of extractive obtained by 60 per cent alcohol is usually from 34 per cent to 44 per cent.

A good deal of powdered gentian is adulterated with either ground olive stones, powdered almond shells, or even pine wood.

A genuine powdered gentian consists chiefly of parenchymatous tissue, most of the cells containing minute crystals, and small oil globules. Only a few starch grains are present. The vessels are scattered and either isolated or in small groups. There are no sclerenchymatous cells or fibres present; this is the most characteristic diagnostic feature of the drug, as most of the adulterants used contain much sclerenchymatous tissue.

Collins ("Chemist and Druggist," 64, 403) has found almond shells and ground pine wood as adulterants, but olive stones are probably more common. These are all easily detected by the microscope. To detect this type of adulterant, the sample is preferably shaken with either water or 70 per cent alcohol and the heavier portion which sinks to the bottom of the water examined. The illustrations on opposite page represent pure gentian and powdered date stones.

Compound Tincture of Gentian.—The characters of this official preparation are given in the table on p. 495.

KINO.

The drug is officially described as the evaporated juice of *Pterocarpus marsupium*. It is probable, however, that it is also obtained from other plants.

Kino is described in the Pharmacopœia as being almost entirely soluble in 90 per cent alcohol, and practically insoluble in ether. Not less than 80 per cent should dissolve in boiling water.

Kino occurs in small angular reddish-black fragments, and sometimes in cakes. The drug is of value solely on account of its astringent properties, and kino-tannic acid is its active constituent.

A genuine kino should not contain more than 15 per cent of moisture and from 1 per cent to 3 per cent of mineral matter.



FIG. 41.—Powdered gentian root.

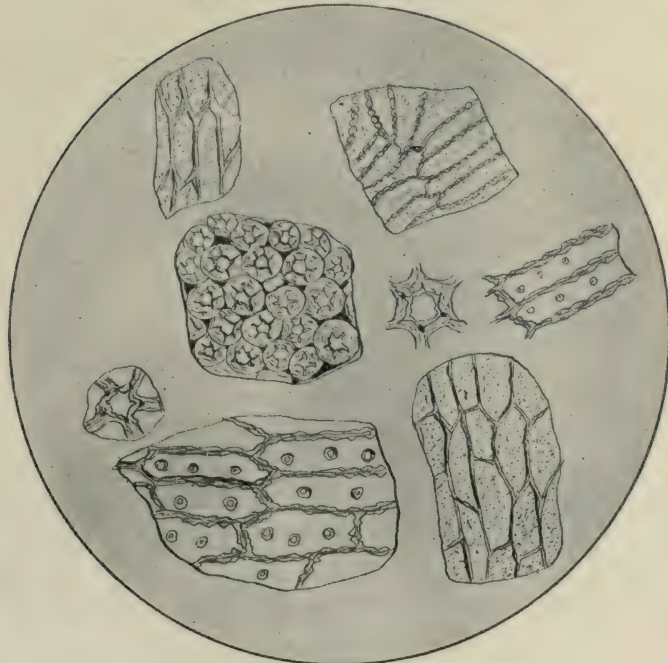


FIG. 42.—Powdered date stones

When determined by the method described on page 11 the tannic acid should vary between 70 per cent and 80 per cent, sometimes even up to 83 per cent.

Tincture of Kino is official. Its characters are given in the table on page 495.

LIQUORICE ROOT.

Liquorice root is official in the British Pharmacopœia, being the drug from which several galenical preparations are made. It is described as the peeled root and subterranean stem of *Glycyrrhiza glabra*, and other species. No standards are given in the Pharmacopœia.

The only methods of analysis available are the determination of the ash, and a microscopic examination.

The ash should vary between 3 per cent and 5 per cent.

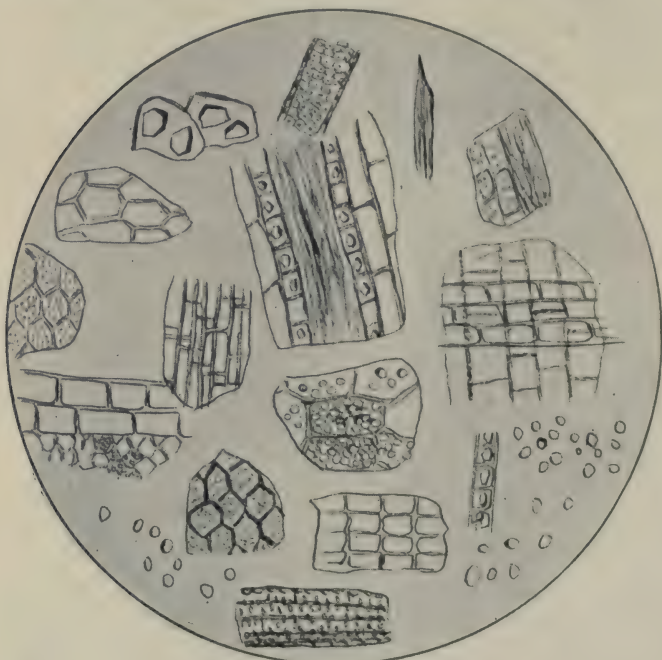


FIG. 43.—Powdered liquorice root.

A microscopic examination of powdered liquorice should reveal numerous parenchymatous cells containing very characteristic starch grains, oval or kidney-shaped, and generally showing a central cavity of the same shape as the grain; numerous pitted vessels and bast fibres are observable. Foreign starchy matter should be looked for and also any very thick-walled cells as may be present through adul-

teration with ground olive stones and similar very much-hardened tissues.

The principal product of liquorice root is liquorice juice, or simply "liquorice". This is the juice of the root, filtered and evaporated to a nearly solid consistency. It is frequently adulterated, either with starch, which is detected by the microscope and by the high amount of substances yielding sugar on inversion. Liquorice juice contains a variable amount of glycyrrhizin, the characteristic substance of the drug, with variable amounts of sugar, gum, starch, and insoluble matter. In examining it, the following determinations are necessary:—

Moisture.—This should not exceed 15 per cent.

Starch and Gums.—Weigh out 2.5 grms. of liquorice juice in a small beaker. Add 15 c.c. hot water, cover with watch-glass, and stand on a hot water bath until thoroughly dissolved, stirring as may be required. Cool. Add 25 c.c. of 80 per cent (by vol.) alcohol, stirring meanwhile. Then add 50 c.c. 95 per cent alcohol with stirring and allow to settle thoroughly, while covered, for about half an hour. Filter through a dry weighed filter. Wash until colourless with 80 per cent alcohol. Dry in water oven to constant weight. This gives the starch and gummy matter.

Glycyrrhizin.—Transfer the filtrate and washings to a flask and distil off the greater part of the alcohol, or until there is only enough liquid for conveniently transferring to a small porcelain evaporating dish. Evaporate to a syrup or to the removal of alcohol, and transfer to a stoppered flask graduated to hold 30 c.c. and make up to the mark with water. Add 3 c.c. of dilute sulphuric acid (10 c.c. conc. H_2SO_4 to 300 c.c. water) slowly and with constant stirring. Allow to stand all night at a temperature of about 60° F. Decant the supernatant liquid, wash the precipitate three or four times with ice water and dissolve in a little dilute alcohol with 2 or 3 drops of ammonia to neutralize traces of sulphuric acid, and evaporate to dryness in a flat-bottomed porcelain dish, till the weight is constant.

B. Hafner ("Zeitschr. des Oesterr. Apoth. Ver." xxxvi. 542) prefers the following method. Ten grms. of the coarsely powdered extract are warmed for several hours with 200 c.c. of 95 per cent alcohol and 25 c.c. of N sulphuric acid, and the insoluble matter is washed with alcohol. The filtrate, made feebly alkaline with ammonia, and diluted with an equal volume of water, is evaporated, made up to 100 c.c. with water and a few drops of ammonia, filtered, and precipitated with dilute sulphuric acid. The precipitated glycyrrhizin is washed with 2 per cent to 3 per cent sulphuric acid, dried in the desiccator, and then extracted with acetone on the water bath. After adding water and barium carbonate, the acetone is expelled on the water bath, the residue digested with 200 c.c. of hot water, and the filtered solution evaporated, dried at 100°, and weighed. The barium glycyrrhizate thus obtained should contain 18.76 per cent of barium, which may be confirmed by evaporating with sulphuric acid, and igniting.

The following method is more rapid and not much less accurate:—

Weigh 2.5 grms. of well-ground juice into a small beaker, cover with 15 c.c. of water and heat on a water bath until dissolved. Cool

and add gradually with stirring 75 c.c. of methylated spirit. Set aside to settle about thirty minutes, filter through a tared paper into an evaporating dish, washing dish and paper with 50 c.c. methylated spirit mixed with 5 c.c. of water. This leaves the insoluble starch and gum on the paper, which is dried and weighed. Bulk the filtrates, evaporate the alcohol off on a water bath. Transfer the syrupy liquid to a cylinder with the aid of 30 c.c. of water, cool strongly in a melting ice bath, and add 3 c.c. H_2SO_4 (5 per cent) with constant agitation, then freeze solid in an ice-salt jacket. If gradually melted the glycyrrhizin forms a compact mass at the bottom of the cylinder. Wash by decantation with about 50 c.c. of H_2O at 0° , drain as far as possible, add 1 c.c. of ammonia, and transfer to a tared dish with absolute alcohol; evaporate and dry at 100° until constant.

Cederberg proposes the following as an accurate method of determining the glycyrrhizin: 10 grms. of the powdered juice are well shaken for an hour in a flask with 200 c.c. of 95 per cent alcohol and 25 c.c. of normal H_2SO_4 . The liquid is filtered and the filter washed with 100 c.c. of hot alcohol. The filtrate is diluted with half its volume of water and rendered alkaline with NH_3 . The liquid is now evaporated to expel alcohol, and made up to 100 c.c. with water, and 100 c.c. of 20 per cent sulphuric acid added when it is cold. The glycyrrhizin is precipitated and allowed to settle, and the supernatant liquid poured off through a filter. The precipitate is then washed with 50 c.c. of 10 per cent H_2SO_4 , again allowed to settle, and the supernatant liquid decanted through the same filter paper. One hundred and fifty c.c. of 90 per cent alcohol is now added to the precipitate and the whole warmed so long as anything will dissolve. This solution of the glycyrrhizin is then filtered through the same paper, which is washed with 50 c.c. of warm alcohol, but the filtrate is not mixed with the previous acid filtrates from the original precipitate. The filtrate is diluted with half its volume of water and rendered neutral with potash solution. It is then made up to 500 c.c. One hundred c.c. is evaporated to constant weight and dried at 110° . A second 100 c.c. is heated and treated with BaCl_2 , and the precipitate filtered on to a tared filter paper, washed with hot water, dried at 110° and weighed. The residue obtained by the evaporation of the 100 c.c. represents the potassium glycyrrhizinate in 2 grms. of juice + K_2SO_4 . The amount of K_2SO_4 is calculated from the amount of barium sulphate found by the precipitation of the second 100 c.c. Thus:—

Residue found in 100 c.c. =	0.5005 grm.	
$\text{BaSO}_4 = 0.385 = \text{K}_2\text{SO}_4$	0.2860	„
Potassium glycyrrhizinate	0.2125	„ (contains 11.58 per cent K)
= glycyrrhizin	0.1884	„ = 9.42 per cent.

Sugars.—Ten grms. of the juice are dissolved with constant stirring in about 100 c.c. of cold water and transferred to a 250 c.c. flask; colouring matter, etc., is precipitated by lead subacetate solution and excess of lead removed by a strong solution of ammonium sulphate. The liquid is filtered and an aliquot part titrated in the usual manner with Fehling's solution. The result is calculated to invert sugar. For the sugars after inversion, 50 c.c. of the filtrate, freed from gum y

matters, etc., by means of alcohol, are inverted with 2 c.c. of strong HCl at 70° for ten minutes, cooled, neutralized and then titrated as usual with Fehling's solution. The difference between this and the former result is calculated to cane sugar if necessary.

Eriksson ("Archiv der Pharm." 1911, 157) proposes the following method, depending on the fact that glycyrrhizin is hydrolysed with the formation of glycyrrhetic acid and glucuronic acid, the latter of which, as it contains an aldehydic residue, reduces Fehling's solution.

Ten grms. are powdered and dissolved in 100 c.c. of water, and 100 c.c. of 90 per cent alcohol added. The mixture is heated on the water bath for half an hour, filtered, the filter washed with 50 c.c. of hot alcohol, the filtrate evaporated until all the alcohol is removed and finally made up to 200 c.c. with water. Forty c.c. of this solution (= 2 grms. of juice) is treated with 25 per cent H_2SO_4 until no further precipitation occurs. After a few hours, the precipitate is filtered through a small filter, and the precipitate washed with 5 per cent H_2SO_4 . The filtrate is reserved for the determination of sugars. The filter and precipitate are transferred to a porcelain capsule and heated for fifteen minutes, with 50 c.c. of 90 per cent alcohol. The liquid is filtered, the filter washed with a little alcohol, and 30 c.c. of water added. The alcohol is evaporated off and 30 c.c. more water added and the glycyrrhizin precipitated with 25 per cent H_2SO_4 . After an hour it is again filtered off. The filter and precipitate are then treated in a porcelain capsule with cold 5 per cent alkali. The solution is filtered into a flask and the filter washed with 100 c.c. of water, and 120 c.c. of Fehling's solution added, and the whole boiled under a reflux condenser for fifteen minutes. The precipitated Cu_2O is collected and weighed in any of the usual methods, and calculated to glucose. The amount of glucose indicated $\times 2.77$ gives the amount of glycyrrhizin.

The filtrate reserved for the determination of sugars is neutralized with 5 per cent alkali and the amount of reducing sugars estimated by the amount of copper oxide precipitated in the cold after standing over night. The saccharose is determined in the filtrate from this by boiling for three minutes with excess of Fehling's solution. Or, alternatively, an aliquot portion may be used for the determination of glucose by reducing boiling Fehling's solution, and the saccharose determined by inverting another portion of the filtrate and determining the total reducing sugar now present.

By the above process, Eriksson finds the following amounts of glycyrrhizin and sugars in typical roots and in pure liquorice juices:—

ROOTS.

	Glucose.	Saccharoses.	Glycyrrhizin.
Italian (dried)	1.39-1.43	2.4-2.57	6.65-7.10
Spanish	1.28	3.20	6.49
Russian	—	6.48	7.70
Russian	traces	6.50	8.15
Russian	3.80	6.25	7.33
" (fresh)	—	2.60	6.72

JUICES.

	Glucose.	Saccharoses.	Glycyrrhizin.	Glycyrrhizin (Cederberg's method).
1.	6.30	11.80	16.45	14.28
2.	3.79	4.52	14.22	—
3.	2.70	8.17	23.90	—
4.	7.82	9.06	12.10	11.10
5.	5.20	11.90	11.59	10.24
6.	5.90	12.48	10.20	9.30
7.	4.50	13.60	9.85	—

Liquorice root varies so enormously according to the country in which it is grown, and even the locality in the same country, that it would be very inadvisable to attempt to lay down any standard figures. Samples must be judged individually and full account taken of their place of origin. The principal variation is in the amount of glycyrrhizin contained in the root, which may be twice as much in a root grown in one district as in one grown elsewhere. This fact divides liquorice juice into three distinct species:—

Firstly, there are what may be described as the ordinary edible juices. These are typified by a glycyrrhizin content of about 10 per cent to 13 per cent, and are sufficiently palatable to be used for the manufacture of stick liquorice. Of these the principal is the Calabrian juice, which forms the basis of nearly the whole of the *pure* stick liquorice of commerce.

Secondly, there are the juices which contain from 17 per cent to 25 per cent of glycyrrhizin, and which are too bitter to be palatable. Of such juices the Anatolian is a type. Juices of this kind form the principal source of supply for the pure block juice and for so many purposes, such as confectionary and the tobacco trades. But they are not made into stick liquorice except with the addition of sugar of some kind or other, when they cannot, of course, be sold legitimately as *pure* liquorice.

Thirdly, there is the sweet Spanish juice which frequently contains 6 per cent or less of glycyrrhizin. This juice has too little "body" to be used much as an ordinary liquorice.

The following analyses are typical of liquorice juice of various origins:—

ITALIAN (CALABRIAN) JUICES (STICK AND BLOCK).

	1	2	3	4	5	6
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Moisture	13.50	12.80	10.95	14.65	11.85	13.6
Ash	6.20	5.98	7.10	6.69	7.55	5.9
Soluble in water	63.90	69.25	63.90	64.80	64.65	65.9
Insoluble in water	22.60	17.95	25.15	20.55	23.00	20.5
Starchy and gummy matter	21.48	20.80	22.80	24.50	26.00	25.2
Glycyrrhizin	9.95	10.18	12.50	11.42	10.50	10.5
Sugars before inversion	12.50	13.50	12.90	13.00	12.00	11.9
Sugars after inversion	15.25	4.95	14.90	15.50	14.70	14.5

ANATOLIAN AND SIMILAR JUICES (BLOCK).

	1	2	3	4
	Per cent	Per cent	Per cent	Per cent
Moisture	18.95	20.50	17.55	16.95
Ash	6.80	6.90	7.22	5.80
Soluble in water	73.55	72.45	75.55	74.55
Insoluble in water	7.50	7.05	6.90	8.50
Starchy and gummy matter	18.61	19.00	17.50	19.65
Glycyrrhizin	23.50	18.75	20.40	21.55
Sugars before inversion	11.50	12.00	10.94	10.88
Sugars after inversion	12.90	13.90	13.20	13.00

SPANISH JUICES (BLOCK).

	1	2	3
	Per cent	Per cent	Per cent
Moisture	9.40	10.50	8.55
Ash	6.50	5.95	7.12
Soluble in water	68.55	65.00	64.90
Insoluble in water	22.05	24.50	26.55
Starch and gummy matter	20.48	21.00	23.50
Glycyrrhizin	6.50	5.95	6.65
Sugars before inversion	14.50	13.09	12.50
Sugars after inversion	15.08	15.25	14.45

The following are analyses of adulterated samples :—

	1	2	3	4	5	6	7
	Pr cent	Pr cent	Pr cent	Pr cent	Pr cent	Pr cent	Pr cent
Moisture	13.50	12.95	12.50	12.90	13.50	14.00	13.2
Ash	3.9	4.7	5.0	4.2	4.6	16.1	4.8
Soluble in water	80.50	78.00	80.56	74.50	77.0	74.60	76.90
Insoluble in water	6.0	9.05	6.94	12.60	9.5	11.4	9.85
Starchy and gummy matter	17.41	16.50	18.00	17.50	16.90	17.05	16.05
Glycyrrhizin	6.40	7.00	7.25	14.25	16.50	8.12	16.00
Sugars before inversion	9.8	12.50	14.00	10.50	18.00	11.5	11.00
Sugars after inversion	24.5	19.5	26.5	18.6	20.50	23.1	19.9

Liquid Extract of Liquorice.—This official galenical is made by exhausting liquorice root with water and adding 25 per cent by volume of 90 per cent alcohol, to the concentrated aqueous liquid when the water has been evaporated until the liquid has a specific gravity of 1.200 at 15°. No official standards are given.

A pure liquid extract of liquorice should have a specific gravity of 1.130 to 1.150: it should contain not less than 39 per cent of solid

matter—often as much as 46 per cent to 48 per cent, and 17 per cent to 18 per cent of alcohol by volume.

Compound Liquorice Powder.—This powder is official in the Pharmacopœia. It consists of:—

Powdered senna	=	2 parts
" liquorice	=	2 "
" fennel	=	2 "
" sulphur	=	1 "
" sugar	=	6 "

The only methods of analysis available are a determination of the ash, which should vary from 4.5 to 5.3 per cent; a microscopic comparison with powder of known authenticity; a determination of the sulphur; the estimation of the matter extracted by 70 per cent alcohol and the amount of sugar. The following analyses are by Evans ("Pharm. Journ." (4) 20, 363):—

	Moisture.	Total Ash.	Insoluble Ash (in H ₂ O).	Soluble Ash.	Extract by Alcohol 70 per cent.	Sugar.	Alcohol Extract, less Sugar.	Sulphur.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	3.86	5.12	2.26	2.86	60.52	50.0	10.52	8.92
2	4.20	6.54	3.98	2.56	60.16	47.7	12.46	8.49
3	3.98	4.84	2.36	2.48	60.08	49.2	10.88	8.91
4	3.68	4.88	2.00	2.88	63.44	50.0	13.44	8.90
5	3.84	4.66	1.68	2.98	63.10	49.6	13.50	8.78

The sugar is determined in the usual manner after inversion with 2 per cent of HCl at 70° for ten minutes, with Fehling's solution.

The sulphur is best determined by heating 1 grm. of the powder with 25 c.c. of strong HNO₃, 5 grms. of KNO₃ and 25 c.c. of water. When oxidation is complete, 25 c.c. of hydrochloric acid are added and the liquid evaporated to dryness in the fume chamber. The mass is then extracted by boiling with 5 c.c. of HCl and 25 c.c. of water, and the insoluble matter washed with water until free from sulphate. The sulphate is then precipitated as BaSO₄ and weighed. From the weight the sulphur is calculated. Not less than 8 per cent is usually obtained in this way, as besides the free sulphur, there are always traces of sulphates present in the mineral matter of the drugs used.

MALE FERN.

The rhizome of male fern, *Aspidium filix-mas* is official in the Pharmacopœia, being used for the preparation of an ethereal extract,

which is known as the liquid extract of male fern. No standards are given for either.

Male fern rhizome should not contain more than 5 per cent of mineral matter.

The active constituent of male fern is filicic acid which either has the formula $C_{35}H_{38}O_{12}$ or $C_{35}H_{40}O_{12}$. It exists both free in the rhizome and also combined in the form of filmarone which slowly decomposes in solution into filicic acid and aspidinol $C_{12}H_{16}O_4$. Amongst the other compounds isolated from the drug are flavaspidic acid $C_{24}H_{28}O_8$ or $C_{24}H_{30}O_8$; albaspidin $C_{25}H_{18}O_8$; and filicinylbutanone $C_{12}H_{16}O_4$.

Kraft ("Zeit. d. Oesterr. Apoth. Verein." xxxiv. 789) regards an amorphous acid which he describes as filicic acid as the only active constituent of the drug, but according to other investigators, several constituents have therapeutic activity as vermifuges.

Kraft recommends the following process for the assay of the ethereal extract:—

Five grms. are shaken for a quarter of an hour with 60 grms. of 95 per cent alcohol and a solution of 2 grms. of potassium carbonate in 40 grms. of water, after which 80 grms. of the mixture are quickly filtered into a separating funnel, and agitated with 50 grms. of ether, 35 grms. of water, and 9 grms. of dilute hydrochloric acid. The ethereal layer, after separation, is washed with another 35 grms. of water, and then slowly evaporated in a 100 c.c. Erlenmeyer flask until only about 2 grms. or less remain. The residue is dissolved in 1.5 gm. of hot amyl alcohol, the solution mixed with 5 grms. of methyl alcohol, the mixture precipitated slowly by the very gradual addition of another 25 grms. of methyl alcohol, and allowed to stand in the stoppered flask overnight in a very cool place. The precipitate is then collected on a tared filter, washed with 10 c.c. of methyl alcohol, and both the filter and flask dried at 60 to 70° C. until the weight is constant. This weight represents the proportion of filicic acid contained in 4 grms. of the extract. Kraft has found the filicic acid in a number of extracts examined to vary between 0.4 and 10 per cent; but he considers that a good extract ought not to contain less than 5 per cent.

MUSK.

Musk, the dried secretion of the preputial follicles of *Moschus moschiferus*, is still official in the Pharmacopœia, although the reason for its inclusion as a drug is not easy to find.

The only standards given are that it should be free from earthy impurities and should yield not more than 8 per cent of ash.

It appears that the grain musk—that is the contents of the sac cut from the animal—is the official drug.

When pure—which is rarely the case—grain musk should yield 50 to 75 per cent to water, and 10 to 12 per cent to 90 per cent alcohol. It should not contain more than 12 to 15 per cent of moisture, nor more than 6 to 8 per cent of mineral matter.

The odoriferous principle of musk is principally a ketone, musk-

one, but as musk is practically entirely used as a perfume material it need not be further discussed.

MYRRH.

This gum-resin is an official drug, being described in the Pharmacopœia as obtained from the stem of *Balsamodendron myrrha* and probably other species. The only official test is that it should assume a violet colour when moistened with nitric acid (distinction from bdellium and false myrrh). The myrrh of commerce, when genuine, is known as Herabol myrrh, but as imported is usually mixed with more or less bdellium and occasionally a little Bisabol myrrh, so that it often requires picking before the absolutely pure gum-resin can be obtained. If a 10 per cent ethereal solution be prepared and a few c.c. evaporated, the residue will at once become deep violet-black on exposure to bromine vapour. The chemistry of myrrh is in a very chaotic condition, and the formulæ assigned to various constituents of it by Tschirch can only be regarded as empirical, even if the constituents themselves have been isolated in a pure condition. It is stated by Tschirch to consist of 50 to 60 per cent of a gum of the formula $C_6H_{10}O_5$; two dibasic resin acids $C_{13}H_{16}O_8$ and $C_{26}H_{32}O_9$ and a resene $C_{26}H_{31}O_2(OH)_3$. About 5 to 8 per cent of essential oil is also present.

O. von Friedrichs ("Archiv Pharm." **245**, 427) gives the following account of the chemistry of myrrh. The resinous portion of the drug after distilling off the volatile oil was extracted with petroleum ether; the portion soluble in that solvent gives acetic acid on destructive distillation. The portion insoluble in petroleum ether, when treated with ether, gave three soluble resin acids, α -, β -, and γ -commiphoric acids; the first two are isomeric, with the formula $C_{14}H_{18}O_4$; γ -commiphoric acid has the formula $C_{17}H_{22}O_5$ and is, therefore, isomeric with myrrholic acid. After saponifying the resin ester a monobasic acid, $C_{28}H_{36}O_8$, commiphorinic acid, was obtained. Two resin phenols were isolated, both containing two hydroxyl groups; they were α -heerabomyrrhol, $C_{18}H_{26}O_5$, and β -heerabomyrrhol, $C_{20}H_{26}O_6$. A monovalent volatile alcohol, $C_{14}H_{22}O_2$, was also liberated by saponification. Heeraboresene was found to have the formula $C_{42}H_{56}O_8$, and to contain a methoxyl group. The resin insoluble in ether contains two acids, α - and β -myrrholic acids, the former has the formula $C_{15}H_{22}O_7$, the latter $C_{25}H_{32}O_6$. Both are monobasic. The gum, which was dextrorotatory, $[\alpha]_D + 23.78^\circ$, afforded mucic acid on oxidation with HNO_3 and furfural on distillation with HCl . It probably contains galactose and arabinose.

The drug when steam-distilled yielded 8.8 per cent of thick, light yellow to greenish, very aromatic essential oil; specific gravity 1.011 at $15^\circ C$; $[\alpha]_D^{20} - 73.86^\circ$. It contains free formic and acetic acids, also a non-volatile crystalline acid with the m.p. $159^\circ C$., which probably exists in the drug as ester. After saponifying the esters another monobasic crystalline acid was isolated, myrrholic acid, $C_{17}H_{22}O_5$, m.p. $236^\circ C$., separating from ether and benzol in small yellow crystals; it is soluble in most solvents, but not in benzol or in petroleum ether. This

is isomeric with the γ -commiphoric acid obtained from the ether-soluble resin. It forms amorphous salts with silver, lead, and copper. The oil contained metacresol, also cuminic and cinnamic aldehydes. By fractionation over sodium under reduced pressure a new tricyclic sesquiterpene, heerabolene, $C_{15}H_{24}$, was isolated. No terpenes were found in the oil distilled by the author, but pinene was found in a commercial sample.

Lewinsohn ("Archiv. Pharm." **244**, 412) describes the essential oil as bright yellow, neutral, and having the specific gravity 0.997 at 20° C., and 1.001 at 15° C.; $a_D - 70^\circ 25'$ at 20° C. Three commercial samples examined were reddish-brown in colour, and more or less acid, the specific gravity was about 1.014, and the a_D ranged from $-40^\circ 3'$ to $-69^\circ 5'$ at 18° C. The characters and constituents of myrrh oil vary with age and method of distillation. Three of the samples contained about 1 per cent of cuminic aldehyde. A fair amount of eugenol and a little metacresol are also present; also pinene, dipentene and limonene; and two sesquiterpenes having the common formula $C_{15}H_{24}$. One has the specific gravity 0.926 at 20°; $a_D + 22.75$, and b.p. 163° C. under 12 mm. The other has the specific gravity 0.911 at 21° C.; $a_D + 30^\circ 4'$; b.p. 151 under 15 mm. They have not been identified with any known sesquiterpenes, although one resembles cadinene. When myrrh oil has been kept it becomes acid and yields acetic and palmitic acids, due to the breaking down of esters.

Myrrh should not contain more than 8 per cent of mineral matter—usually from 5 to 6 per cent. The amount soluble in alcohol (90 per cent), water, and petroleum ether may be determined, and also the acid and ester values. These figures should be in accordance with the following which were obtained by the author on six samples of myrrh freed from all extraneous gum resins:—

1. Soluble in alcohol . . .	33.8	41.9	38	37.5	36	43
2. Soluble in water . . .	29.5	31.2	37	40.5	38.5	34
3. Soluble in petroleum ether . .	19.6	20.1	17.5	18.5	20.8	16.5
Acid value of (1) . . .	59	68	66	70	72	66.4
Ester value of (1) . . .	108	121	117	131	119	124
Acid value of the myrrh . . .	20.5	27	26	28	23	20.5
Ester value of the myrrh . . .	34	48	45	50	43	50

The nitric acid test for myrrh is, according to Greenish, best applied to the ethereal or petroleum-ether extract ("Pharm. Jour. 1901, II. 666). The extract is allowed to stand in an inverted dish over the fumes of nitric acid when it gradually acquires a violet coloration. Alcoholic solutions of myrrh such as the tincture are best diluted with water and the dilute emulsion extracted with petroleum ether, and the test applied to the residue left after evaporation of the petroleum ether. Bisabol myrrh and bdellium do not give the reaction. Bromine water or vapour gives a similar reaction, but not so well-marked.

Tucholka ("Year Book of Pharmacy," 1898, 180) gives the follow-

ing test for Bisabol myrrh. A solution of 1 part of the sample in 15 of petroleum ether and 3 parts of glacial acetic acid is made, and 6 drops of this are cautiously mixed with 3 c.c. of strong H_2SO_4 . In the presence of Bisabol myrrh a rose-red coloration appears at the juncture of the liquids, and the whole of the acetic acid layer soon acquires a red colour. With genuine myrrh, only a slight red colour is acquired by the acid layer, whilst the line of contact is dull green.

Tincture of Myrrh is an extract of 4 ounces of myrrh by alcohol (90 per cent) sufficient to produce 20 fluid ounces of the tincture. It should have the following properties:—

Specific gravity . . .	0.848	to 0.858
Solid residue . . .	4	„ 6 grms. per 100 c.c.
Alcohol by volume . . .	84	„ 86 per cent

It is to be noted that, as no official standard exists for the percentage of matter soluble in alcohol, in the gum resin, it is difficult to condemn samples containing less than 4 per cent of solid residue. Tinctures, however, prepared from a good myrrh will contain fully 5 per cent of solid residue.

PEPSINE.

The British Pharmacopœia describes pepsine as an enzyme obtained from the mucous lining of the fresh and healthy stomach of the pig, sheep, or calf. It should dissolve 2500 times its weight of hard-boiled white of egg when tested as follows:—

If 12.5 grms. of coagulated firm white of eggs, 125 c.c. of water containing about 0.2 per cent of HCl, and 0.005 gm. of pepsine be digested together at 105° F., for 6 hours, with frequent shaking, the coagulated albumen dissolves leaving only a few small flakes, in an almost clear solution. The white of eggs should be prepared by boiling quite fresh eggs for fifteen minutes, cooling, removing adhering water with a towel, and at once rubbing the white through a sieve having twelve meshes per centimetre, and at once using the product. Pepsine is also required to be soluble in 100 parts of alcohol (90 per cent).

Pepsine occurs as a powder or in scale form. Good specimens are always pale in colour. It should not be very hygroscopic, otherwise the presence of peptones is indicated. It should have no odour, and should always be of faint acid reaction.

Many samples of commercial pepsine are mixed with sugar of milk or powdered starch. Such samples will not satisfy the requirements of the British Pharmacopœia, and should not be sold as pepsine without qualification. Many of them are honestly reduced to a standard strength, when the original pepsine is found to possess a very high dissolving power on albumen. The following analyses represent the composition of average samples of pepsine:—

	A. H. Allen.	Parry.
	Per cent	Per cent
Moisture	5.00	4.00 to 6.5
Pepsine (true)	61.02	62.706 „ 69.55
Peptones	5.29	2.54
Mineral matters	1.00	0.9 „ 1.87

The examination of pepsine is almost confined to the determination of its value as a solvent of albumen, but when a complete analysis is required, it is rarely necessary to do more than determine the moisture, the ash, and the total nitrogen. If peptone is suspected, the solution may be precipitated with zinc sulphate, and the filtrate from this again precipitated with bromine. The nitrogen found in the bromine precipitate multiplied by 6.3 will give the approximate amount of peptones. The moisture should not exceed about 5 to 6 per cent, and the mineral matter should not be more than 1 to 1.75 per cent.

If not soluble in water and alcohol, the samples should be tested for starch with iodine, and for sugar of milk in the usual manner.

In attempting any assay of pepsine for its proteolytic value, it is to be remembered that the conditions of the experiment are very important, as the formation of peptones eventually retards the action of the enzyme. Hence different conditions of experiment will cause greatly different results to be obtained.

Hercod and Maben, have, in a report presented to the 1910 International Congress of Pharmacy at Brussels, made an exhaustive comparative study of the methods of pepsin assay of the principal Pharmacopœias. They have examined the methods official in the following authorities; the Belgian, British, German, Italian, Swiss and United States Pharmacopœias, and the French Codex, the following being the quantitative requirements of each of these authorities in reference to the assay process.

Pharmacopœia.	Acidity of Digestive Solution per cent HCl abs.	Proportion of Acid to Pepsin per cent.	Temperature of Digestion deg. C.	Duration of Digestion in Hours.	Preparation of Albumin.		Standard. 1 Pepsin digests Albumin.
					Egg-boiled Minutes.	Sieve-meshes per cm.	
Belgian . . .	0.25	250	40	1	10	10	100
British . . .	0.2	5000	40.5	6	15	12	2500
German . . .	0.125	125	45	1	10	10	100
Italian . . .	0.09	90	38.40	1 to 2	—	—	100
Swiss . . .	0.2	200	40	1 to 2	5	15	100
United States .	0.3	3600	52	2½	15	16	3000
French . . .	0.25	150	50	6	—	—	25 (fibrin)
Standard proposed by authors .	0.25	250	52	2	10	15	2000

Hercod and Maben recommend the following method :—

Take coagulated white of egg (obtained by boiling fresh eggs for ten minutes), pass through a No. 40 sieve, and press between two sheets of filter-paper to remove surplus moisture; weigh 10 grms., and place it in a flask of 200 c.c. capacity, containing 100 c.c. of distilled water previously heated to 52°C ., 0.25 per cent absolute HCl, and 5 c.c. of a 0.1 per cent solution of pepsin. Place the flask in a water bath at 52°C ., and digest at that temperature for two hours, stirring gently every fifteen minutes with a rotatory movement by means of a glass rod. At the expiration of two hours the albumin should be dissolved, the solution having an opalescent appearance.

To get a true idea of the value of pepsine, it is not sufficient to determine the amount of albumen dissolved, but also the amount of peptone which has been produced. Weak samples of pepsine may dissolve a large quantity of albumen but may only convert it into syntonin, whereas a strong pepsine will carry the digestive process further, and convert it all into peptones. Again, the colloidal nature of the substance causes the action to take place at the surface where the pepsine meets the albumen, no penetration taking place. So that the finer the particles of albumen the greater the dissolving action. Still further, even when albumen in an experiment appears not to be dissolved, it is usually in an advanced stage of digestion so that it is difficult to estimate the digestive action if any albumen remain undissolved. It is therefore best to arrange experiments so that the end of the time reaction corresponds with the solution of the whole of the albumen. Bartley ("American Druggist and Pharmaceutical Record," Oct. 1893) has described a process which in the author's experience gives exceedingly good results. He eliminates the varying nature of egg albumen by using a solution which contains the whites of several fresh eggs instead of the coagulated albumen, and tests the liquid at regular intervals to see if conversion is complete. His process is as follows:—

Solution No. 1.—Take the whites of several fresh eggs, mix them thoroughly, and to 100 grms. of the mixed egg albumen add 900 c.c. of distilled water, or in this proportion if smaller quantities are used. Mix the solution well, and heat from three to five minutes. After cooling, make up the mixture with water to the original volume. The liquid may be strained, if necessary, through fine muslin; but if the eggs are fresh only a slight coagulum will form during the heating, and will yield a slightly opalescent liquid, containing 10 per cent of white of egg. As the latter contains, on an average, about 12.2 per cent of dry albumin, 100 c.c. of this liquid will contain 10 grms. of egg-white, or 1.22 grms. of dry albumen.

Solution No. 2.—Weigh out 1 gm. of the pepsin to be tested, add 25 c.c. of water, and then add 2 c.c. of diluted hydrochloric acid. Now add water enough to make the solution up to 50 c.c., or if it be a high-grade pepsin make up to 100 c.c. after adding 4 c.c. of diluted acid.

Procedure.—Measure out into a beaker or bottle 50 c.c. of the albuminous liquid, and warm in a water bath to 35° to 40°C . (95° to 104°F). Now add to this solution 2 c.c. of diluted hydrochloric acid, and from one-half to five c.c. of the pepsin solution. The more active the pepsin, the less the quantity to be taken. In the valuation

of high-grade pepsins it is best to use 100 c.c. of albumen solution, containing 10 grms. of egg-white, and 1 c.c. of pepsin solution containing 0.010 gm. of pepsin. It may sometimes be necessary, with an unknown pepsin, to perform a preliminary test to determine the approximate time before spending too much time on an accurate test. It is best to so regulate the quantity of pepsin and albumen that the time shall be about two hours.

The time when the pepsin is added must be carefully noted, and the temperature of the solution must be kept between 35° and 40° C. (95° to 104° F.). At intervals of ten minutes, after the first hour, draw out a few drops of the solution with a nipple pipette (dropper), and float it upon a small quantity of pure nitric acid in a conical minim glass. The digestion is incomplete as long as a white zone of coagulated albumen appears at the line of contact of the two fluids. Note the time when the nitric acid ceases to give this coagulation. This end-reaction can generally be easily determined. In this manner three elements in the calculation of the digestive power of the pepsin are obtained, viz. :—

The weight of the egg-albumin, A,

The weight of the pepsin taken, P,

The time consumed, T.

As regards a standard time, the author fixes upon three hours as the average time of stomach digestion. The relation between the quantities of albumen and pepsin is expressed by the fraction $\frac{A}{P}$, i.e. it is found by dividing the amount of albumen (5 grms. in the above directions for weaker pepsins) by the amount of pepsin used when 1 c.c. of the solution above mentioned is taken for the test, viz. .02 gm. This would give the amount of albumen digested by 1 part of pepsin in the observed time of the experiment as 250 grms. But the time is not the standard time. Assume that the time required for the digestion was two hours. The relation of this to the standard time, three hours, would be $\frac{3}{2}$. The above result must then be multiplied by this ratio in order to give the amount of albumen capable of being digested in the standard three hours. Expressed in the form of an algebraic equation we have : D (digestive power) $= \frac{A}{P} \times \frac{3}{T}$, and substituting the above values :—

$D = \frac{5}{.02} \times \frac{3}{2} = \frac{15}{.04} = 375$ grms., showing that 1 gm. of this pepsin is capable of digesting 375 grms. of egg-albumen in three hours, or 750 grms. in six hours.

As egg-white contains about 12.2 per cent of dry albumen, 1 gm. of this pepsin will digest 45.75 grms. of dry albumen in three hours, or 91.5 grms. in six hours.

The advantages claimed for this process over other methods are :—

1. The shorter time consumed.
2. Uniformity in results.
3. The avoidance of the necessity for shaking the solution during digestion.

4. A more exact statement of results.

5. The weaker solution of albumen used causes less interference with the action of the pepsin by the peptone formed.

Stebbing ("Analyst," xiv. 197, 210, 229) recommends the process suggested by Kremel. Egg albumen in scales is dried at 40° and powdered. One gram. is treated in a 100 c.c. flask with 0.1 gram. of the pepsine and 50 c.c. of 0.2 per cent HCl. The liquid is kept at 40° for three hours. It is then neutralized with alkaline carbonate, heated to 90° C., and cooled after coagulation is complete. The liquid is then made up to 100 c.c. and 50 c.c. filtered off and evaporated to dryness. This residue represents the albumoses and peptones formed and is probably the truer measure of the digestive power of the pepsine, than any experiment which determines the amount of albumen dissolved, of which much is only converted into syntonin. From the weight of the peptones, etc., thus determined, a deduction must be made for the amount of mineral matter present, and also for the amount of pepsin in solution, which may be determined by a blank experiment without the albumen.

Allen ("Analyst," xxii. 258) prefers the following process. About 1 gram. of egg albumen in scales is powdered and treated with 20 c.c. of water in a 100 c.c. flask. When it is dissolved the liquid is heated in a water bath to coagulate the albumen and cooled to 40° C. 0.1 gram. of the pepsine is then added and also 25 c.c. of decinormal hydrochloric acid. The liquid is then warmed to 40° C. for three hours. The liquid is neutralized by sodium carbonate solution, and is then heated to 90° C. for ten minutes. It is then cooled, made up to 100 c.c., and filtered. The precipitate consists of syntonin and any unaltered albumen, while the filtrate contains peptones and albumoses.

Fifty c.c. of this latter are saturated with zinc sulphate, allowed to stand for half an hour, with occasional agitation, and then filtered. The precipitate is washed with cold saturated solution of zinc sulphate, and the filtrate made up to 250 c.c. with water slightly acidulated with HCl, and the filtrate treated with excess of bromine water.

The albumoses may be calculated from the amount of nitrogen in the zinc sulphate precipitate and the peptones from that in the bromine precipitate (see under Extract of Meat, p. 405). An allowance must be made for the amount of nitrogen present in the pepsine used.

The proportion between the amount of albumen dissolved and the amount of true peptones and albumoses formed is very small, and it must be remembered that mere solution processes are rather a measure of the amount of syntonin formed than of peptones.

CANADA TURPENTINE.

This oleo-resin, better known as Canada balsam, is official in the Pharmacopœia. It is an oleo-resin obtained from *Abies balsamea*. It is officially required to solidify when mixed with about one-sixth part of its weight of magnesia moistened with a little water.

Canada balsam contains about 25 per cent of an essential oil con-

sisting almost entirely of terpenes, principally *lævo*-pinene. This oil is *lævorotatory* and boils at 160° and almost completely distills below 170° . The balsam also contains about 60 per cent of resin acids, which have been described under the names *canadinic acid* $C_{18}H_{34}O_2$; *canadolic acid* $C_{19}H_{28}O_2$; and *canandinic acid* $C_{19}H_{30}O_2$. An indifferent *resene*, *canadorestene* $C_{21}H_{40}O$, has also been isolated, and occurs to the extent of about 6 per cent.

Canada balsam should have the following characters:—

Specific gravity at 15°	0.985 to 0.995
Refractive index	1.5200 (about)
Essential oil	20 to 25 per cent
Optical activity	$+1^{\circ}$ to $+5^{\circ}$
Optical activity of essential oil	<i>lævorotatory</i>
Acid value	70 to 90
Ester value	4 „ 15
Acid value of the oil-free resin	100 „ 120

Colophony is a frequent adulterant, mixed with ordinary turpentine oil. Such mixtures will generally give higher acid values, and the essential oil may be *dextrorotatory*.

BURGUNDY PITCH.

This resin is officially described as the resinous extract of the stem of *Picea excelsa*.

It consists principally of *pimaric anhydride*, with a small amount of essential oil.

The analytical examination of this substance is not well understood, but genuine samples examined by the author show that it should have the following characters:—

Acid value	130 to 145
Ester value	under 20
Iodine value	about 120 to 130

It should be soluble in twice its weight of glacial acetic acid.

Many samples of so-called Burgundy pitch consist merely of common rosin, pitch, and turpentine. Most of these are not completely soluble in twice their weight of glacial acetic acid.

LIQUID TAR.

Under the name *Pix liquida*, the bituminous liquid obtained by the destructive distillation of *Pinus sylvestris* and other species of pine, is official in the British Pharmacopœia. It is known commercially as Stockholm tar.

The official requirements are that its specific gravity should be from 1.020 to 1.150. If it be shaken with water, the water acquires an acid reaction and gives a red colour with dilute ferric chloride solution. It should be completely soluble in 10 volumes of 90 per cent alcohol.

Genuine Stockholm tar should be soluble in an equal volume of absolute alcohol, ether, or chloroform, and almost entirely soluble in

3 volumes of 5 per cent solution of potash. It is completely soluble in 96 per cent acetic acid, which distinguishes it from other tars except beechwood tar. If 1 volume be well shaken with 5 volumes of petroleum ether and the petroleum separated and shaken with an aqueous solution of cupric acetate (0.1 per cent), the petroleum acquires a green colour, due to the formation of soluble copper salts of the tar acids. Beechwood tar does not give this reaction.

RESIN.

Resin or colophony is an official drug. It is the residue left after the distillation of the oil of turpentine (q.v.) from the crude oleo-resin of various species of *Pinus*.

The official requirements are that it should be soluble in 90 per cent alcohol, ether, benzol, and carbon disulphide, and that it should leave no appreciable ash.

The bulk of the resin of commerce is obtained from the crude American turpentine, but French turpentine yields a very high-grade product also.

The chemistry of this resin is in an unsettled state, but it is clear that it consists of several resin acids either isomeric or closely related. It is generally agreed that abietic acid is the typical resin acid present. The probable formula for this acid is $C_{20}H_{30}O_2$ and it is possibly identical with sylvic acid. Some investigations tend to support the theory that the acids are present as anhydrides, but the ready solubility in alkaline solution is against this hypothesis. At all events from the analytical point of view, it is certain that resin consists almost entirely of abietic or closely allied acids, with traces of esters and up to 5 per cent of neutral resins or resenes. Pure resin should have the following characters:—

Specific gravity at 15°	1.070 to 1.085
Acid value	150 „ 182
Ester value	5 „ 20
Iodine value	118 „ 130
Unsaponifiable matter	about 6 „ 8 per cent
Specific rotation in alcohol . .	+ 58° „ + 68°

The very low price of resin makes it more interesting as an adulterant than anything else—for, after the examination of many hundreds of samples, the author has never found one adulterated.

The following details of the characteristics of resin, therefore, will be of interest:—

A valuable reaction for colophony, suggested by Liebermann, but modified by Storch and Morawski, consists in treating the substance with acetic anhydride, cooling the liquid, and separating the acetic anhydride. Sulphuric acid of 1.5 specific gravity is then allowed to flow gently into the tube containing the acetic anhydride, when a reddish-violet colour will be immediately produced at the junction of the two liquids if colophony be present. The colour soon changes to reddish-brown.

It is frequently necessary to separate fatty acids and resin acids.

This is best done by the following method which is due to Twitchell and Gladding.

About 5 grms. of fatty and resin acids are boiled with excess of alcoholic potash for half an hour under a reflux condenser. The alcohol is then evaporated, the residue dissolved in water, and unsaponifiable matter removed by agitation with ether. The aqueous liquid is separated and acidified with hydrochloric acid. The separated acids are removed by shaking with ether; the aqueous acid solution is neutralized, evaporated to about 25 c.c., re-acidified, and shaken out with ether. After distilling off the ether from the united ethereal extracts, the residue of resin and fatty acids is dissolved in 50 c.c. of absolute alcohol, and the fatty acids converted into esters by passing a moderately rapid current of dry hydrochloric acid gas through the solution cooled by ice-water to a temperature not above 10° C. When the operation is complete (which is usually the case in from one to two hours), the liquid is allowed to stand for half an hour at the ordinary temperature. It is then diluted with five times its volume of water, and boiled under a reflux condenser for half an hour. The cool solution is agitated with several successive quantities of ether until the extracts are colourless. The aqueous liquid is neutralized, evaporated to 50 c.c., acidified and repeatedly extracted with small quantities of ether to recover the water-soluble constituents of colophony. The mixed ethereal solutions are shaken out with about 50 c.c. of a solution containing 10 grms. of caustic potash, 10 grms. of alcohol and 100 c.c. of water, when a brown layer usually separates out between the ether and the alkaline solution and is drawn off with the latter. This layer contains a considerable portion of the resin-soap, which is only slightly soluble in the potash solution. The ether is shaken with water to remove soluble resin-soaps; then with two successive quantities (10 c.c.) of the potash solution; and finally with water until the washings are colourless. The alkaline liquid is now acidified and agitated with ether until completely extracted. The acid solution is neutralized, evaporated to a small bulk, re-acidified, and again shaken out with ether. The total ether extracts are washed with 20 c.c. of water, and the ether distilled off. The residue of resin acids so obtained—still contaminated with unchanged fatty acids—is treated with several small successive additions of absolute alcohol to remove the last traces of water, and weighed. The fatty acids still remaining in the resin-acids are removed by Gladding's process. From 0.4 gm. to 0.6 gm. of the resin-acids, obtained as above, should be placed in a 100 c.c. stoppered and graduated cylinder, and dissolved in 20 c.c. of 95 per cent alcohol. A drop of phenol-phthalein solution is added to the alcoholic solution, and then concentrated caustic soda solution (1 of NaOH to 2 of water) until the reaction is just alkaline. The loosely-stoppered cylinder and its contents are heated for a short time in the water bath, then cooled, and ether added up to the 100 c.c. mark. One gm. of dry powdered silver nitrate is added, and the contents of the cylinder are shaken for fifteen minutes to convert the fatty and resin acids into silver salts.

When the insoluble salts have completely settled (preferably after

standing overnight), 70 c.c. of the solution should be pipetted into a second 100 c.c. cylinder and shaken with 20 c.c. of dilute hydrochloric acid (1 : 2). The ethereal layer is drawn off, and the aqueous liquid twice shaken with ether. The united ether extracts are washed with water, filtered, and the ether distilled off. The residue, amounting to about 10 c.c., is evaporated, dried for a short time at 100° to 115° C., and weighed. The weight of the resin acids so found is calculated back into the first weight (impure acids) obtained, and then on the original substance taken. The percentage found is corrected by the subtraction of 0.4 per cent, this allowance being made for a small amount of unesterified fatty acid, which is always present. As colophony contains an average of 8 per cent of unsaponifiable matter, a second correction is necessary, the true percentage of colophony in the substance under examination being found by the following equation, in which the corrections are combined :—

$$100 \frac{\text{percentage of resin acids found} - 0.4}{92} = \text{percentage of colophony.}$$

A useful method for separating the resin acids (which represent 90 per cent of the colophony) from other resins which yield silver salts insoluble in ether, such as shellac, etc., is that used by the author for shellac analysis. The process is as follows :—

About 0.5 gm. of the sample is dissolved in the smallest possible quantity of alcohol and the solution cautiously treated with alcoholic potash till it is just neutral to phenol-phthalein. This solution, containing the potash salts of the acids, together with the neutral constituents of the sample, is poured into about 100 c.c. of water contained in a separator, and about 0.5 gm. of silver nitrate, dissolved in a little water, added. The acids are precipitated as silver salts, and on shaking the liquid twice with ether, the silver salts of the resin acids of colophony are completely dissolved, while the silver compounds of the other acids remain insoluble. The ethereal solution is filtered, repeatedly agitated with water to remove silver nitrate, dilute hydrochloric acid added, and the liquid well shaken. The silver salts are decomposed, silver chloride being precipitated, and the resin acids recovered by evaporating the washed ethereal solution to dryness.

THUS.

Thus, or gum thus as it is known commercially, is the so-called American frankincense. It is an official oleo-resin scraped from the trunk of *Pinus palustris*.

No official standards exist.

As a matter of fact, thus is collected from other species of *Pinus*, and so far as the author can ascertain is nothing other than crude turpentine from which a portion of the essential oil of turpentine has evaporated, leaving a crude concrete oleo-resin containing rather less essential oil than the oleo-resinous turpentine. Genuine samples examined by the author were treated to drive off the small quantity of oil of turpentine, and the residues were then found to be indistinguishable analytically from common resin, or colophony.

SCAMMONY

Scammony (Scammonium of the British Pharmacopœia) is a natural gum resin obtained by incision of the living roots of *Convolvulus scammonia*. The root itself is also official in the Pharmacopœia, and contains the following:—

	Per cent
Resin	6 to 9
Extractive matter	12 „ 15
Starch	7 „ 8
Mineral matter	9 „ 13

The pure resin of scammony prepared from the dried root is also official as scammony resin. This should be entirely soluble in 90 per cent alcohol and practically free from ash.

The principal constituent of scammony is scammonin $C_{34}H_{56}O_{16}$ which is closely related to or probably identical with the glucoside of tampico jalap. It is a glucoside, melting at at 131° and having a specific rotatory power -23° .

Pure scammony, i.e. the crude gum resin obtained naturally, forms masses of varies sizes of a brown, dark grey or nearly black colour. It forms a grey powder when pulverised, which should yield only the slightest reaction for starch, and should contain, according to the Pharmacopœia, at least 70 per cent of resin soluble in ether and not more than 3 per cent of ash. An alcoholic solution should not afford a blue colour with solution of ferric chloride. No other Pharmacopœial standards are given.

A genuine scammony will certainly rarely contain less than 70 per cent soluble in ether when powdered and extracted in a Soxhlet tube, but pure samples will often contain as much as 6 per cent or even 7 per cent of mineral matter. The greater part of the scammony of commerce is grossly adulterated, especially the so-called Aleppo scammony. Starchy matter is the principal adulterant, which is detected under the microscope, or by testing the cooled aqueous decoction with iodine; chalk is sometimes present, which will raise the ash value, and will cause a little of the powdered drug to effervesce with dilute hydrochloric acid. Lead sulphide is occasionally found. The resin, extracted by means of ether (or preferably a mixture of 85 per cent of ether of specific gravity 0.735 and 15 per cent of 90 per cent alcohol) should be examined, and should have the following characters:—

Acid value	14 to 21
Ester value	200 „ 225
Iodine value (Hübl)	10 „ 15

Taylor ("Amer. Journ. Pharm." 1909, **81**, 105) has examined a number of genuine scammony resins and of the so-called "Mexican" scammony resin which is obtained from the root of *Ipomœa Orizabensis*. He gives the following figures:—

	Resin per cent.	Acid Value.	Ester Value.	Iodine Value.
True scammony	8.1	21.1	211.3	13.3
" "	7.93	15.5	222.5	10.8
" "	8.06	15.6	219.8	13.0
" "	7.71	18.2	221.7	14.3
" "	8.52	18.8	218.1	14.6
Mexican scammony	16.75	15.5	171.1	8.7
" "	16.83	21.5	165.6	11.5

According to Guignes ("Bull. Soc. Chem." 1908 [iv.] 3, 872) the specific rotation of scammony resin affords a means of detecting certain adulterants. He gives the following values for alcoholic solution :—

Scammony resin extracted from the gum resin, $[\alpha]_d$	up to $-24^{\circ} 30'$
" " " " roots	$-18^{\circ} 30'$ to $-23^{\circ} 30'$
Tampico jalap resin	$-34^{\circ} 20'$
Orizaba jalap resin	$-24^{\circ} 45'$
Jalap resin	$-30^{\circ} 10'$ to -36°
Colophony	$+6^{\circ}$ to $+7^{\circ}$
Sandarac	$+31^{\circ}$ to $+34^{\circ}$
Mastic	$+29^{\circ} 30'$
Guaiacum resin	-17°

The author has examined numerous samples of Mexican scammony root and finds that it contains from 14 to 20 per cent of resin. The ester value is a most valuable method of discriminating between the genuine resin and that from Mexican scammony, and as many tons of Mexican root are imported annually into this country, it is necessary to carefully examine samples of the resin, many commercial specimens of which are made entirely from Mexican root.

These characters will ensure the absence of colophony, and guaiacum resin is detected by the blue colour imparted to an alcoholic solution by ferric chloride solution.

If the presence of guaiacum resin be proved, its amount can be determined approximately by an estimation of the methoxy value of the resin. Pure scammony resin has a value of 0 to 2, whilst guaiacum resin gives a figure of 72 to 85.

This value is determined in the following manner: About 0.3 gm. to 0.4 gm. is treated in a glycerine bath at 120° to 140° with 10 c.c. of hydriodic acid (1.70 specific gravity) in a flask of 40 c.c. capacity, connected with three bulbs, the first being empty, the second containing water, and the third water with red phosphorus in suspension. After passing through the bulbs, which absorb hydriodic acid and iodine, the alkyl iodide is absorbed in a flask containing 5 c.c. of a 40 per cent aqueous solution of silver nitrate and 50 c.c. of alcohol; a second flask with half the quantities of silver nitrate and alcohol may be added as a precaution. The mixed silver solutions are rendered acid with dilute nitric acid, and the silver iodide filtered off and weighed. The weight of silver iodide multiplied by 0.132 gives the amount of methoxyl CH_3O , the methoxyl number

indicating the number of milligrams of CH_3O in 1 grm. of the substance.

SENNA.

Two varieties of senna leaves are official in the Pharmacopœia, those of *Cassia acutifolia*, known as Alexandrian senna; and those of *Cassia angustifolia*, known as East Indian or Tinnivelly senna. No standards are given.

The constituents of senna are, in spite of very numerous investigations, but poorly understood. There appear to be present bodies either isomeric or identical with emodin, iso-emodin and chrysophanic acid. These are known as senna-emodin, etc., and are probably the result of the decomposition of glucosides, which are usually present in small quantity in the dried leaves, although the greater portion of these has decomposed.

The only available means of examining senna are the determination of the ash, and a microscopic examination.

The ash of senna leaves usually varies between 9 per cent and 14 per cent. If a higher ash be found in a powdered senna, it may still be genuine, but is probably of very inferior quality, containing much "siftings". The ash should be almost entirely soluble in hydrochloric acid.

Greenish describes the microscopic characters of the powder as follows:—

The powder exhibits fragments of epidermal tissue consisting of polygonal cells and bearing stomata and hairs or the scars of fallen hairs. Each stoma is enclosed between or bordered by two cells, arranged parallel to it; the hairs are one-celled, thick-walled and warty. It also exhibits groups of sclerenchymatous fibres, which, however, should not be present in excessive quantity.

In addition to these characters, characteristic rows of cells with prismatic crystals are to be found. An examination of the sample side by side with the powder of an authentic specimen will enable most possible adulterants to be detected easily.

According to Sayre ("Amer. Jour. Pharm." 1896, 585) the characters of the hairs enable one to distinguish between the two varieties of leaves.

Alexandrian senna is more hairy than Indian, a mixture of equal parts of the two in No. 60 powder containing ten hairs of the former to one of the other variety. The hairs of Alexandrian senna have a sharp curve near the base, while those of Indian senna are straighter, shorter, and stouter. Almost invariably the hairs of both kinds remain unbroken when the drug is powdered, and these distinctions may therefore be of practical value. The epidermal cells also differ in form, those of Indian senna being somewhat smaller and more uniform in size, whilst the angles are more acute than in the Alexandrian variety. Careful measurement of the cells gives the following average results: Indian senna, 35 micromillimetres in diameter; Alexandrian senna, 40 micromillimetres.

Compound Tincture of Senna is an official drug, the characters of which will be found in the table on p. 496.

Compound Mixture of Senna, or black draught, is also an official preparation of senna. It is a mixture of liquid extract of liquorice, compound tincture of cardamons, aromatic spirit of ammonia, infusion of senna, and sulphate of magnesium. The usual adulterant is an excess of its cheapest ingredient, magnesium sulphate. A genuine mixture should have the following characters:—

Solid residue	15 to 16.5 per cent
Alcohol by volume	9.0 „ 9.5 „
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	25 grms. per 100 c.c.

AROMATIC SPIRIT OF AMMONIA.

This official drug is an aromatic alcoholic solution of ammonia and ammonium carbonate.

The official requirements for the drug are as follows:—

Specific gravity 0.888 to 0.893. Twenty c.c. require 25.5 c.c. of normal sulphuric acid for neutralization, corresponding to about 2.4 per cent of NH_3 , or 2.16 grms. in 100 c.c. Twenty c.c. after the addition of 16 c.c. of a solution of barium chloride (10 grms. per 100 c.c.), should yield a precipitate which becomes more copious on heating to 71°C ., and after filtering, the filtrate should yield a further precipitate when more of the barium chloride solution is added, and the liquid is again heated.

White (*"Pharm. Journ."* 4, x. 144-148) has shown that the statements in the British Pharmacopœia in reference to the strength of the solution of ammonia directed to be used in this preparation, are incorrect. The Pharmacopœia states that ammonia solution, of specific gravity 0.891 contains 32.5 per cent of NH_3 . He has shown that 31.6 per cent or a figure very near to this is the true value. He also shows that, whereas the Pharmacopœia requires ammonium carbonate to be of such strength that 1 gm. should require at least 18.7 c.c. of normal H_2SO_4 for neutralization, no samples are to be found which require more than 18.2 c.c. From these facts it follows that the calculated alkaline value given by the Pharmacopœia, viz. that 20 c.c. should require 25.5 c.c. of normal H_2SO_4 for neutralization is incorrect, and samples must be made with rather more ammonia than directed, in order to come up to the official requirements.

The barium chloride test has been shown to be quite unreliable and has been criticized severely by White and by F. C. J. Bird.

It has been shown that any quantity between 18 and 23 c.c. of the spirit will behave as directed when 20 c.c. are used according to official directions. By the addition of a little ammonium chloride, the reaction is, as pointed out by Bird, rendered reliable. He recommends the test to be carried out as follows:—

To 20 c.c. of the aromatic spirit of ammonia add 5 grms. of ammonium chloride, agitate vigorously, and add 16 c.c. of solution of barium chloride. Warm to 160°F ., cool to normal temperature, and filter. The filtrate, on the addition of more barium chloride and warming gives no further precipitate. Should a slight

opalescence be produced by the barium chloride it should disappear completely on heating, but any precipitate of barium carbonate would remain permanent. Twenty-one c.c. of aromatic spirit of ammonia tested under exactly similar conditions will be found to yield a precipitate on the further addition of barium chloride, which does not disappear on warming. The reaction appears to be quite complete at the time of filtering, as is evident from the following table:—

16 C.C. BARIUM CHLORIDE SOLUTION.

Spt. Am. Ar. taken.	Filtrate on Standing.	BaCl ₂ to Portion of Filtrate.	(NH ₄) ₂ CO ₃ to Portion of Filtrate.
20 c.c.	No ppt.	Faint opalescence which disappears on warming.	ppt.
21 c.c.	No ppt.	Permanent ppt. on warming and allowing to stand for ten minutes.	No ppt.
22 c.c.	No ppt.	Copious ppt.	No ppt.

In examining this drug, it must be remembered that a deficiency in alkaline strength of from 1 per cent to 5 per cent of the total necessary, may be due to the fact that, in the manufacture of the spirit, the directions of the Pharmacopœia have been minutely followed, the deficiency being due to the errors in that authority. If the spirit be of full strength, the directions of the Pharmacopœia have probably not been literally adhered to.

SPIRIT OF NITROUS ETHER.

This drug is officially directed to be made by distilling a mixture of alcohol, sulphuric and nitric acids, and copper.

The official requirements for the drug are as follows:—

Specific gravity 0·838 to 0·842. If the spirit be poured on to a layer of acid solution of ferrous sulphate (10 per cent) a deep olive-brown colour is produced at the surface of contact of the liquids. Ten c.c., mixed with 5 c.c. of normal soda solution, and 5 c.c. of water should become yellow, but should not turn brown on standing for twelve hours (limit of aldehyde). It should not effervesce, more than very faintly, when shaken with sodium bicarbonate. One volume agitated briskly at intervals during five minutes in a brine-charged nitrometer, with 1 volume of 10 per cent solution of potassium iodide and 1 volume of dilute sulphuric acid should yield at normal temperature and pressure, and when freshly prepared, from 6·25 to 7 volumes of nitric oxide gas, corresponding to 2·5 per cent of ethyl nitrite. And even after it has been kept some time and the vessel containing it has been occasionally opened, it should yield not much less than five times its volume of gas, corresponding to nearly 2 per cent of ethyl nitrite, or a minimum of 1·75.

A more unscientific and unsatisfactory standard than the above

would be very difficult to invent. It necessarily fixes the amount of 1·75 per cent of ethyl nitrite as the bottom limit, and so long as the sample is of this strength no exception can be taken to it, in spite of the standard for freshly prepared spirit which is 2·5 per cent.

Spirits of nitrous ether, or sweet spirit of nitre as it is now also termed alternatively in the Pharmacopœia, is a liquid of very complex composition.

It contains ethyl nitrite, alcohol, aldehyde, ethyl acetate, ethyl nitrate, free acids, and water. Various other compounds are also present in traces.

The literature of this drug is voluminous, the greater part of it being devoted to proving that it is very unstable and is very liable to deterioration. This is well recognized to be true, and the majority of successful prosecutions for selling this drug below its proper strength are certainly due to the deterioration of an originally genuine article.

MacEwan ("Pharm. Journ." 3, xiv. 817) has given the following figures, which show the effect of keeping on the drug :—

	Ethyl Nitrite.	HNO ₂	Acetic Acid.	Aldehyde.
	Per cent	Per cent	Per cent	Per cent
(1) B. P. Spirit (old) . . .	0·87	0·47	1·20	0·80
(2) " " (1 week old)	3·54	0·22	0·21	0·85
" " (2 " ")	—	0·26	0·25	0·95
" " (3 " ")	3·14	0·27	0·35	—
(3) B. P. " (2 days old)	2·01	—	—	0·80
" " (4 " ")	—	0·24	0·22	1·14
" " (7 " ")	1·24	0·32	0·25	2·00
(4) B. P. " (1 month . old) . . .	1·93	0·24	0·41	1·67
(5) London Pharmacopœia (4 months old) . . .	3·53	0·16	0·29	1·50
(6) London Pharmacopœia (4 months old) . . .	1·64	0·35	0·49	1·43
(7) London Pharmacopœia (4 months old) . . .	0·22	0·19	0·25	0·20

The principal factors which cause the amount of ethyl nitrite to be reduced on keeping are the traces of water present, which cause decomposition to be rapid; exposure to air, light and excessive temperature.

Although the standards laid down for this drug must be adhered to for official purposes, it is a fact that very carefully prepared samples may have a specific gravity up to 0·848 or even 0·850. No exception would be taken to this, so long as the amount of ethyl nitrite is maintained.

Free Acid.—The amount of free acid is determined, as recommended by MacEwan, in the following manner :—

Ten c.c. of the sample are placed in a flask with a drop of phenolphthalein solution, and a few drops of solution of methyl-orange are

added. A porcelain slab is also spotted with drops of methyl-orange solution. Semi-normal soda solution is run in until the pink colour of the acid solution and methyl-orange begins to change, when a drop is removed by a glass rod and brought into contact with a spot of methyl-orange solution. If the spot assumes a pink tint the *nitrous acid* is not quite neutralized, in which a little more alkali is run in until a spot of methyl-orange is rendered only faintly pink. The amount of alkali used is noted, and the titration continued until neutrality is indicated by the pink colour of the phenol-phthalein. Each c.c. of semi-normal alkali used for producing neutrality to methyl-orange = 0.0235 grm. of HNO_2 ; and each c.c. of additional alkali used is equivalent to 0.030 grm. of acetic acid. The results are sufficiently approximate for all practical purposes.

Aldehyde is best determined by Thresh's colorimetric process. Ten c.c. of the sample are diluted with 20 c.c. of water and 3 c.c. of a saturated solution of caustic soda added. The mixture is heated to boiling-point for a few seconds, then cooled and after two hours is diluted with 20 c.c. of warm alcohol (free from aldehyde) and made up to 60 c.c. with water. The liquid is quite clear and of a reddish-yellow colour. As the colour soon alters, it is best to immediately make a solution of potassium bichromate to match the colour. A solution of aldehyde containing 1 per cent of aldehyde in pure alcohol is then treated in the same manner and the colours of the two matched by dilution of the one having the deeper colour as in the process of Nesslerizing. The amount of aldehyde present is thus calculated with approximate accuracy.

Estimation of Ethyl Nitrite.—Eykmán's process is a most accurate one for the determination of ethyl nitrite. The following, according to A. H. Allen, is the most reliable method of carrying out this process:—

Take a small flask A, tubular in shape with a round bottom, and insert a tight-fitting rubber-stopper, through which passes a narrow glass tube B. This tube should extend nearly to the bottom of the flask terminating in a turned-up point to prevent any gas from entering. The rest of the tube outside the flask should be bent over and joined to a long, narrow vertical tube by an india-rubber joint. This tube should also terminate in a point, and when placed in a conical glass D should nearly reach the bottom. From one side of the flask should branch a tube E which is connected with the stopper of a Lunge's nitrometer G by means of a few inches of india-rubber F. Take a solution of soda of about 1.10 specific gravity specially prepared for the experiment by being previously shaken with a small quantity of ferrous sulphate, thus ensuring freedom from dissolved oxygen, and allowing the precipitated oxide of iron to subside. Use a solution of ferrous sulphate containing 100 grms. of the powdered crystallized salt in 500 c.c. of water with 0.5 c.c. of strong sulphuric acid.

Pour about 30 c.c. of the iron solution into the flask. Wet the india-rubber cork well and insert firmly in the neck. Then connect with the nitrometer, which contains a small quantity of soda solution in the cup, and see that the tap of the nitrometer is closed. The glass D should contain the solution of iron into which the tube C

must be immersed. The screw-clip at H must be left open. Heat the flask to expel the air through C, then remove the flame and allow about 30 c.c. of iron solution to enter the flask, firmly closing the clip at H. Heat the contents of the flask to boiling. When the india-rubber at F begins to swell, open the tap at G, and allow the air from the flask to bubble through the soda solution in the cup of the nitrometer. Close the tap G when all the air has been expelled, remove the flame and allow the contents of the flask to cool. Put 5 c.c. to 10 c.c. of the sample (according to its strength) in the glass D with 10 c.c. to 20 c.c. of water containing 1 or 2 grms. of common salt. Then

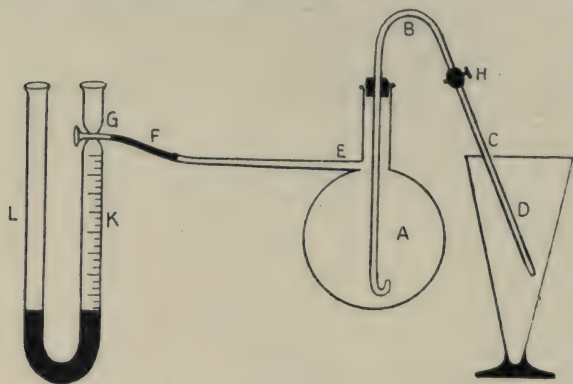
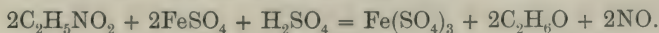


FIG. 44.—Eykman's apparatus for nitrous ether.

very carefully open the clip F and allow the liquid to flow into the flask until the opening of tube C is covered. Pour a little iron solution into glass D, also 5 c.c. of the dilute sulphuric acid, and allow this to pass into the flask. Continue to do this until the glass and tube have lost their brown colour, at the same time being careful not to allow any air to enter the flask. Heat the contents of the flask to boiling, having previously closed the clip at H. Turn the tap G, to open connexion between the graduated tube K of the nitrometer and the flask, as soon as the india-rubber joint F shows signs of pressure. This reaction produces nitric oxide gas, which passes into K where it is collected. As soon, however, as the contents of the flask are no longer brown, the tap G is closed and the clip at H opened simultaneously, thus forcing back the liquid into A. The apparatus is then ready for another experiment. When the liquid has had time to assimilate the temperature of the air, i.e. in about half an hour, notice the volume of gas in the nitrometer, being careful that the level of the liquid in the tube L is identical with that in K.

The following formula expresses what has occurred :—



The percentage of ethyl nitrite found in the volume of nitric oxide obtained can be calculated as follows, when *v* represents the number

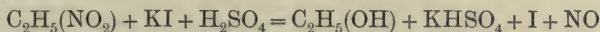
of c.c. of gas obtained, p the barometric pressure in mm., e the tension of aqueous vapour at the temperature at which gas is measured, d the density of the sample (water = 1); n the number of c.c. employed; and t the temperature in centigrade degrees:—

$$\text{C}_2\text{H}_5\text{NO}_2 = \frac{v}{d \times n} \times \frac{p - e}{273 + t} \times 0.1207.$$

When it is not necessary to have strictly accurate results, omit the corrections for pressure, temperature, and tension of aqueous vapour. The calculation will be much simpler. If the volume of 0.030 grm. of nitric oxide (representing 0.075 grm. of $\text{C}_2\text{H}_5\text{NO}_2$) under the ordinary conditions of pressure and temperature be taken at 23.55 c.c., then

$$\frac{\text{volume of gas in c.c.} \times 0.3184}{\text{measure of sample in c.c.} \times \text{density of sample}} = \text{percentage by weight of } \text{C}_2\text{H}_5\text{NO}_2.$$

The official process with potassium iodide and dilute sulphuric acid is, however, quite accurate enough for most purposes. The nitrometer should be charged with a saturated salt solution and the end immersed in the same liquid. Five c.c. of the spirit should be placed in the cup and carefully drawn in, avoiding the inclusion of any air bubbles. Five c.c. of a 10 per cent solution of potassium iodide is then drained in, and this is followed by 5 c.c. of dilute sulphuric acid. It is advisable to place about 6 c.c. of the latter in the cup and leave a little therein, as this will guard against the possibility of air bubbles being drawn in, which would now be unnoticed on account of the presence of nitric oxide in the top of the nitrometer. The reaction is as follows:—



from which the amount of ethyl nitrite can be calculated. The nitrometer should be read off at about 15° C., after adjusting the levels of the liquids inside and outside by lowering or raising the nitrometer as may be found necessary.

Dott ("Pharm. Journ." 3, xv. 492) has proposed titrating the iodine liberated in the above reaction by a solution of sodium thio-sulphate, but if this be done in the open air, the process is useless, whilst if it be done in a confined space, the measurement of the gas is at least as accurate as, and more rapid than, the titration process.

Muter ("Analyst," iv. 125) has published a process based on the oxidation of the ethyl nitrite by means of permanganate of potassium, but as this process includes other oxidizable bodies, such as aldehyde, and is more tedious than processes giving more accurate results it need not be described here.

If the sample contains free nitrous acid, this will, in Eykman's and similar processes, yield nitric oxide gas. So that if absolutely accurate results are required, the amount of free nitrous acid as indicated by titration, as described above, must be multiplied by 1.59 and subtracted from the apparent amount of ethyl nitrite found. The Pharmacopœia does not differentiate between ethyl nitrite and other nitrous

compounds, but guards against more than traces of nitrous acid by the test with sodium bicarbonate.

Methyl alcohol compounds should be searched for, in case methylated spirit should have been used in the manufacture of the sample. Fifty c.c. should be dehydrated with ignited potassium bisulphate or carbonate and the dehydrated spirit poured off. Fifteen c.c. or 20 c.c. are distilled with 10 grms. to 12 grms. of dry calcium chloride, from a *water bath*, until practically nothing more comes over. Five c.c. of water are then added to the flask and another 2 c.c. distilled. This 2 c.c. is then tested for methyl compounds in the following manner:—

Two grms. of potassium bichromate, and 2·5 c.c. of concentrated H_2SO_4 are mixed in a small distilling flask with 20 c.c. of water and the 2 c.c. of distillate to be tested. After standing for fifteen minutes the mixture is distilled, and when 20 c.c. have passed over, the acid distillate, which contains formic acid if methyl alcohol were present, is treated with a slight excess of sodium carbonate, evaporated down to 10 c.c., and enough acetic acid added to give the liquid a distinct acid reaction. 0·1 grm. of silver nitrate dissolved in 3 c.c. of water is then added and the whole heated to 80° for a few minutes. In the presence of methyl alcohol, a precipitate of brown or brownish-black metallic silver is formed, and a thin film of silver is deposited on the tube. A slight darkening may be neglected.

Liquor Ethyl Nitritus is also an official preparation of ethyl nitrite, slightly stronger than the *spirit*, and much more stable. Its official characters are as follows: Specific gravity 0·823 to 0·826. It should not effervesce when shaken with sodium bicarbonate. Ten c.c. when mixed with 5 c.c. of normal caustic soda solution and 5 c.c. of water should not turn yellow (absence of aldehyde). It should yield, when tested in a nitrometer, as in the case of the *spirit*, at least 7·6 times its volume of nitric oxide (when freshly prepared); or at least 6·33 times its volume after it has been kept for some time.

SQUILLS.

The dried bulbs of *Urginea scilla* are official under this name. No standards are given.

This drug contains several glucosides of which scillitoxin is probably the most active; scillipicrin and scillin are also present. All of these require investigation, but for such details of their chemistry as are known, reference should be made to a paper by Merck ("Pharm. Journ." 3, ix. 1038). Scillain is probably a non-toxic glucoside but is better defined chemically than the others. Its formula is $(C_6H_{10}O_3)_x$. On hydrolysis it yields dextrose, butyric acid, isopropyl alcohol, and bodies not investigated. Squills should yield from 2·5 per cent to 4 per cent of ash on incineration.

Tincture of Squills.—The characters of this preparation are given in the table on p. 496.

Vinegar of Squills.—This drug is an extract of squills by acetic acid containing 4·27 per cent of acetic acid. No standards are given.

A small quantity of acetic acid is lost during the process of making,

so that the resulting vinegar will not contain quite as much acid as is used in its preparation. The amount of extractive from the squills also slightly lessens the percentage of acid. It has been alleged that this drug loses its acidity somewhat rapidly by keeping, but this is not the case. The following are figures which cover *all* properly prepared samples :—

	Freshly Prepared.	One Month Old.	Twelve Months Old.
Acetic acid	Per cent 3·6 to 4·1	Per cent 3·5 to 4	Per cent 3·4 to 3·9

This drug formed the subject of the well-known appeal case of *Hudson v. Bridge*, which is dealt with fully in Vol. II.

Oxymel of Squills.—This official drug is a mixture of an acetic acid extract of the squills, and of clarified honey.

The only standard given is a specific gravity of 1·320.

Lucas ("Pharm. Journ." iv. 17, 778) has made a careful examination of a number of samples of oxymel of squills and gives the following details. As most commercial honey is lævorotatory, it follows that oxymel of squills will also be lævorotatory. Even if a genuine, slightly dextrorotatory honey be used, squills contain a lævorotatory sugar, which will more than neutralize the dextrorotation of the honey. If a dextrotatory sample be found it is almost certainly made with an adulterated honey, containing glucose; or with cane sugar. The presence of glucose is, apart from the dextrorotation, revealed by the following tests :—

One volume of the sample is mixed with 4 volumes of water and filtered through animal charcoal, the filtrate being returned until nearly colourless. It is divided into two portions. To one is added 5 volumes of absolute alcohol. Genuine honey gives only a slight opalescence, while an opaque precipitate forms at once if glucose be present, in more than small amount. To the second portion of filtrate is added 1 drop of 10 per cent iodine solution. Pure honey is unaffected; if glucose be present the iodine is at once bleached, as the former rarely contains less than 0·05 per cent, and frequently as much as 0·1 per cent of sulphurous acid. If more iodine be added, drop by drop, the slightest excess gives rise to a reddish-brown colour due to the amylo- and erythro-dextrins present. Honey containing glucose is strongly dextrorotatory.

Lucas gives the following figures for authentic samples, Nos. 1-8 and No. 10. Nos. 9 and 11 are adulterated with glucose.

The sugar values were determined on a Ventzke scale polarimeter, using 65·12 grms. of oxymel of squills (i.e. 2·5 times the normal sugar weight) diluted with water, cleared with lead subacetate, alumina cream, and sodium sulphate and made up to 250 c.c. and then filtered through animal charcoal.

Density at 15.5° C.	Gm. of Real Acetic Acid in 100 c.c.	Alcohol Test.	Iodine Test.	Cupric Reducing Power.	Direct Reading.	After Inversion.
(1) 1.310	1.92	Nil	Nil	49.1	-15.5	-20.4
(2) 1.323	1.87	Nil	Nil	53.2	-16.3	-21.6
(3) 1.336	1.08	Nil	Nil	56.1	-15.7	-19.1
(4) 1.326	2.46	Nil	Nil	53.0	-11.0	-14.8
(5) 1.318	1.06	Nil	Nil	49.2	-15.1	-16.7
(6) 1.322	0.36	Nil	Nil	55.4	-14.0	-15.7
(7) 1.327	0.53	Nil	Nil	52.3	-12.1	-16.9
(8) 1.303	1.20	Nil	Nil	53.2	-13.9	-15.7
(9) 1.325	1.05	{ heavy ppt.	{ deep red brown	45.6	+27.4	+23.9
(10) 1.350	0.26	Nil	Nil	57.5	-12.1	-17.4
(11) 1.321	1.81	{ heavy ppt.	{ deep red brown	46.2	+26.4	+23.6

These, calculated to cane sugar, invert sugar and glucose, are as follows:—

No.	Percentage reckoned as Sucrose.	Percentage reckoned as Invert Sugar.	Percentage as Glucose.
1	3.6	48.5	Nil
2	3.9	53.4	—
3	2.5	55.6	—
4	2.8	51.8	—
5	1.18	48.9	—
6	1.25	54.3	—
7	3.5	51.5	—
8	1.3	52.3	—
9	2.57	36.1	19.1
10	3.9	56.4	—
11	2.06	36.8	18.8

STORAX.

Storax is a balsam obtained from the trunk of *Liquidambar orientalis* apparently as a pathological secretion only arising after damage to the bark or wood. [American storax is a different substance, known as sweet gum, and is obtained from *Liquidambar styraciflua*. The so-called *Styrax calamitus* is probably the powdered bark of one of the species of *Microstemon* from which the bulk of the balsam has been expressed, mixed with more or less sawdust.]

The crude drug is not official in the British Pharmacopœia, which only recognizes "prepared storax," which is made by dissolving the crude drug in alcohol, filtering and evaporating the solvent. The official requirements for this drug are that it should contain no moisture, and when boiled with a solution of potassium bichromate and sulphuric acid it evolves an odour resembling essential oil of bitter almonds.

Storax contains styrol $C_6H_5CH:CH_2$; cinnamic acid, cinnamyl cinnamate, phenyl-propyl cinnamate, ethyl cinnamate, traces of vanillin, and lesser known resin alcohols, esters, and hydrocarbons.

The principal adulterants of storax are colophony and fatty oils. Storax after driving off water is soluble to the extent of 95 per cent in 90 per cent alcohol, the remainder being principally mechanical impurities. The examination of this drug should commence with the determination of the amount soluble in 90 per cent alcohol. The residue should be examined and if it be of an oily nature, it should be quantitatively saponified. A high saponification value of this residue indicates the presence of a fatty oil, and the fatty acids can be separated from the saponification liquor in the ordinary way.

Fatty oils are indicated by the behaviour of the *dried* storax with petroleum ether. This solvent has little effect on pure dried storax, but in the presence of the fatty matter usually employed as an adulterant it becomes quite milky. Another simple method for the detection of fatty adulterants is as follows: Mix 4 grms. of the undried storax with 9 mgs. of absolute alcohol, make up to 40 mgs. with 90 per cent alcohol (at about $20^{\circ} C.$) and shake the liquid for a few minutes. After filtering, pour into a flask and place in cold water or, better still, on ice for about six hours. In the presence of much fatty matter a flocculent precipitate will make its appearance.

Crude storax should not contain more than 30 per cent of water, and 1 per cent of ash.

The "purified storax" thus prepared by solution in alcohol should then be examined, and should have the properties of purified storax of the Pharmacopœia.

Purified storax should be entirely soluble in 90 per cent alcohol. It should have a specific gravity between 1.110 and 1.123. The acid value of the purified balsam should be between 75 and 90. The ester value varies from 120 to 140, although these limits are sometimes exceeded.

Colophony may be detected by extracting the storax with petroleum ether. A genuine storax will rarely give more than 45 per cent of extract and this will have an acid value not exceeding about 60. Storax adulterated with colophony will give a high amount of extract, and this will have an acid value up to over 100. The ester value of the extract from pure storax will be over 140. This figure is reduced by the addition of colophony.

TEREBENE.

Terebene is an official drug which is described as a mixture of dipentene and other hydrocarbons, obtained by agitating turpentine oil with sulphuric acid until it becomes optically inactive, and then distilling the oil in a current of steam.

The official tests are as follows: specific gravity 0.862 to 0.866. It is optically inactive: it distils between 156° and 180° and should leave only a slight viscid residue. Not more than 15 per cent should distil below 165° .

Very few commercial samples comply strictly with these requirements, and it is probable that no sample can be made to exactly satisfy the official standards. Very few samples are quite optically inactive, and the unreasonableness of the requirement in this direction is shown by the fact that however near to optical inactivity the product is, on fractional distillation nearly every fraction will be found to be optically active. Further, it is impossible to make a sample of terebene which boils entirely below 180° . All samples will contain up to 5 per cent or 7 per cent boiling about 180° . Oils made from French turpentine more nearly meet the requirements of the Pharmacopœia.

A terebene may be considered satisfactory if it possesses the following characters: Specific gravity 0.862 to 0.866; optical rotation not exceeding 1° either way; not more than 5 per cent boiling below 160° ; not more than 8 per cent boiling above 185° ; not more than 2 per cent of viscid residue (which generally results from the oxidization during evaporation). A normal sample will give results approximating to the following, on fractionation (Tyrer and Wertheimer, "Pharm. Journ." 1900, II. 101).

Fraction.	Sp. Gr.	Rotation (186 mm.).	Refractive Index.
150 . .	0.8753	+2.8	1.46207
150-164 .	0.8766	+1.4	623
164-169 .	0.8754	+1.1	584
169 . .	0.8733	+0.8	603
169-169.5 .	0.8832	+0.5	603
169.5-169.7	0.8807	+0.4	652
169.7-169.9	0.8835	+0.4	702
169-170 .	0.8968	0	771
170-170.5 .	0.8942	0	672
170.5-171 .	0.8892	-0.1	683
171 . .	0.8855	-0.4	702

The following tables represent the principal characters of a number of important galenical preparations of the British Pharmacopœia:—

STANDARDS FOR TINCTURES OF THE BRITISH PHARMACOPŒIA.

In most Cases not Official.

Name of Tincture.	Specific Gravity at 15° C.	Solid Residue Gr. per 100 c.c.	Alcohol by Volume.	Gr. per 100 c.c. Active Ingredient.
			Per cent	
Tinct. Aconiti890 to .895	1.3 to 1.6	66 to 68	0.025 to 0.065
" Aloes970 " .980	6.5 " 7.6	38 " 42	—
" Arnice893 " .899	0.65 " 0.8	67 " 69	—
" Asafœtidæ910 " .918	9 " 10	60 " 63	—
" Aurantii recentis875 " .885	1.6 " 1.9	72 " 76	—
" Belladonnæ910 " .915	0.5 " 0.65	57 " 58	0.048 to 0.052
" Benzoin Co. . .	.890 " .904	18 " 20	75	—
" Buchu925 " .935	2.9 " 4.0	56 to 58	—
" Calumbæ915 " .925	0.8 " 1.2	56 " 58	—
" Camphoræ Co. . .	.913 " .923	0.3 " 0.37	57 " 59	0.43 to 0.49
" Cannabis Indicæ845 " .850	3.5 " 4.2	85 " 87	—
" Cantharidis835 " .840	0.15 " 0.17	89 " 90	—
" Capsici890 " .898	1 " 1.2	68 " 69	—
" Cardamomi Co. . .	.945 " .955	6 " 7.2	52 " 54	—
" Cascarillæ895 " .902	2 " 2.5	64 " 67	—
" Catechu978 " .984	13 " 16	50 " 53	—
" Chirata920 " .925	1 " 1.2	57 " 58	—
" Chloroform et Morphinæ Co. . .	1.010 " 1.015	29 " 30	51 " 52	—
" Cimicifugæ922 " .928	1.2 " 1.5	57 " 58	—
" Cinchonæ914 " .924	6.2 " 6.9	63	0.95 to 1.05
" " Co. . .	.914 " .924	4.6 " 5.2	65	0.45 " 0.55
" Cinnamomi900 " .905	1.5 " 2.2	65 to 67	—
" Cocci950 " .960	2 " 2.5	42 " 44	—
" Colechici Sem. . .	.950 " .960	1.9 " 2.4	41 " 43	0.05 to 0.09
" Conii895 " .902	1.3 " 1.45	66 " 68	0.05 " 0.1
" Croci925 " .930	2.2 " 2.9	56 " 58	—
" Cubebæ840 " .845	1.2 " 1.5	83 " 86	—
" Digitalis980 " .985	2.9 " 3.7	54 " 56	0.4 to 0.75
" Ergotæ Ammon. . .	.935 " .942	2.8 " 4.0	50 " 52	—
" Ferri Perchloridi . . .	1.085 " 1.088	12	22	—
" Gelsemii920 " .928	1.2 to 1.3	56 to 57.5	0.02 to 0.03
" Gentianæ Co. . .	.961 " .970	4.0 " 5.5	42 " 43	—
" Guaiaci Ammon. . .	.898 " .907	14 " 17.5	69 " 71	—
" Hamamelidis947 " .954	1.4 " 2.0	44 " 45	—
" Hydrastis923 " .929	2.0 " 2.5	56 " 58	0.4 to 0.6
" Hyoscyami950 " .960	2.3 " 3.6	43 " 44	0.008 " 0.015
" Iodi875 " .880	—	84 " 86	—
" Jaborandi956 " .959	2.6 to 4.3	42 " 43	0.08 to 0.15
" Jalapæ910 " .915	3.5 " 4.7	65 " 66	1.45 " 1.55
" Kino995 " 1.000	20 " 24	49 " 53	—
" Krameriæ935 " .940	4.5 " 5.0	54 " 56	—
" Lavandulæ Co. . .	.835 " .840	0.45 " 0.52	88 " 89	—
" Limonis875 " .885	1.4 " 1.5	76 " 77	—
" Lobeliæ Æthereæ812 " .817	0.9 " 1.5	—	.02 to .04
" Lupuli935 " .943	3.5 " 4.0	50 " 54	—
" Myrrhæ848 " .858	4 " 6	84 " 86	—
" Nucis Vomice910 " .915	1.7 " 1.8	60 " 61	0.24 to 0.26
" Opii955 " .962	3.4 " 3.7	42 " 44	0.7 " 0.8
" " Ammon. . .	.894 " .901	2.7 " 2.9	62 " 64	0.1 " 0.12
" Podophylli844 " .848	3.4	86	3.3 " 3.5
" Pruni Virg. . .	.931 " .938	2.3 to 2.8	53 to 55	—
" Pyrethri900 " .905	1.5 " 1.9	67 " 69	—
" Quassiæ945 " .950	0.2 " 0.5	43 " 45	—
" Quillaiæ920 " .927	1 " 1.4	55 " 58	—
" Quininæ885 " .893	3.5 " 3.9	74	1.634

STANDARDS FOR TINCTURES OF THE BRITISH PHARMACOPŒIA.

In most Cases not Official.

Name of Tincture.	Specific Gravity at 15° C.	Solid Residue Gr. per 100 c.c.	Alcohol by Volume.	Gr. per 100 c.c. Active Ingredient.
Tinct. Quininae Ammon.	·925 to ·930	1·8	Per cent 54	1·471
" Rhei Co. . . .	·970 " ·975	12 to 13	49 to 50	—
" Scilla	·962 " ·970	10·5 " 12·5	51 " 52	—
" Senegae	·935 " ·944	4·2 " 5·0	54 " 55	—
" Sennae Co. . . .	·985 " ·995	8 " 9	38 " 40	—
" Serpentariae	·895 " ·900	1·4 " 2·0	66 " 68	—
" Stramonii	·953 " ·962	3·2 " 4·0	42 " 43	0·02 to 0·03
" Strophanthi	·894 " ·897	0·4 " 0·7	68·5 " 69	0·05 " 0·08
" Sumbul	·900 " ·905	2·2 " 2·8	65 " 67	—
" Tolutanae	·862 " ·870	8·5 " 9·0	81 " 84	—
" Valerianae Ammon.	·940 " ·945	2·7 " 3·7	49 " 51	—
" Zingiberis	·840 " ·845	0·3 " 0·4	87 " 89	—

STANDARDS FOR FLUID EXTRACTS OF THE BRITISH PHARMACOPŒIA.

In most Cases not Official.

Liquid Extract.	Specific Gravity.	Extractive (Gm. per 100 c.c.).	Average Alcoholic Strength per cent by Vol.)	Gr. of Ac- tive Ingredi- ent per 100 c.c.
Extract. Belladonnae liq. .	0·890 to 0·920	11 to 14	66 to 69	—
" Cascarae liq. . . .	1·054 " 1·066	24	19	0·75
" Cimicifugae liq. . . .	0·875 " 0·890	10	78	—
" Cinchonae liq. . . .	1·115 " 1·130	38 to 43	11 to 13	5·0
" Cocae liq. . . .	0·995 " 1·031	18 " 20	49 " 52	0·2 to 0·6
" Ergotae liq. . . .	1·005 " 1·025	12 " 15	30 " 32	—
" Glycyrrh. liq. . . .	1·130 " 1·150	40	17·5	—
" Hamamelidis liq. . . .	1·025 " 1·040	21	34	—
" Hydrastis liq. . . .	1·025 " 1·040	20 to 24	36 to 40	4 to 6 total alkaloids)
" Ipecac. liq. . . .	0·885 " 0·915	9 " 12	78 " 79	2 to 2·25
" Jaborandi liq. . . .	1·020 " 1·050	21 " 22	33 " 35	0·2 " 0·75
" Nucis vomicae liq. . . .	0·945 " 0·965	11 " 12·5	61 " 63	1·5
" Opii liq. . . .	0·985 " 0·995	3 " 3·1	18	0·7 " 0·8
" Pareira liq. . . .	1·025 " 1·050	19	22	—
" Sarsae liq. . . .	1·055 " 1·085	26	19	—
" Taraxaci liq. . . .	1·045 " 1·060	24	20	—

THE CONCENTRATED LIQUORS OF THE BRITISH PHARMACOPŒIA.

A number of concentrated liquors are official in the Pharmacopœia, having been introduced as official representatives of a class of concentrated preparations known as concentrated infusions which although commanding extensive employment, are not official. These concen-

trated preparations do not, on dilution, exactly represent the freshly prepared infusions, hence the choice of the name "concentrated liquors" by the Pharmacopœia authorities.

The following are the average characters of these galenicals:—

Liquores.	Specific Gravity.	Extractive (Gm. per 100 c.c.)	Alcoholic Strength (by Vol.)
Liquor. Calumbæ conc. .	0.987 to 0.997	3.5 to 6	18 to 20
" Chirata conc. .	0.978 " 1.000	3.8 " 5.5	18 " 19
" Cuspariæ conc. .	1.008 " 1.020	8 " 10	18 " 19
" Krameriæ conc. .	1.007 " 1.015	8 " 9	18 " 19
" Quassia . .	0.976 " 0.990	0.25 " 0.5	18 " 19
" Rhei . .	0.998 " 1.035	10 " 13	17 " 18
" Sarsæ Co. conc. .	1.020 " 1.045	9 " 10	18 " 19
" Senegæ conc. .	1.010 " 1.032	10 " 14	21 " 22
" Sennæ conc. .	1.000 " 1.080	12 " 16	17 " 18
" Serpentariæ conc.	0.990 " 1.005	5 " 5.5	18 " 19

It must be borne in mind, however, that in the case of liquor calumbæ concentratus, the official formula is so unsatisfactory that the finished product may, unless prepared on a small scale, have characters outside the above limits.

It has been stated that the characters of the finished product depend on the power of the press used for expressing the liquid from the macerated drug, and as pointed out by F. C. J. Bird, this statement has been practically investigated by A. C. Abraham, who showed that when using a hand-press the finished liquor had a specific gravity of 1.029 and extractive 2.16 per cent, whilst with a powerful hydraulic press these figures became respectively 1.032 and 3.66. An inspection of the formula even suggests this result, for in the first instance (hand-press) the volume of the expressed liquid being smaller, and the quantity of spirit added remaining the same, the proportion of alcohol in the mixture is greater, and precipitation consequently is more copious. Loss of extractive therefore follows both from the diminished amount in the pressings and the larger quantity precipitated by the spirit; there is also much loss of alcohol owing to the greater alcoholic strength of the liquid absorbed by the filter paper and the more voluminous precipitate. There is no compensation for this in the official process, as the deficiency of the volume is directed to be made up by addition of water to the filtrate.

The formula for liq. calumbæ conc. is one which furnishes a preparation, varying greatly according to the conditions of manufacture, both in extractive and percentage of alcohol, a low proportion of the latter bringing in its train a continuous deposition of sediment from the development of acidity in the liquid.

As the non-official "concentrated infusions" are still used to a very large extent, being diluted with seven times their volume of water when the fresh infusion is prescribed, it will be as well to give the following figures which represent a large number of samples of commercial products. These figures are those obtained in the author's

laboratory. It may be pointed out that even if the amount of extractive matter be 8 times that of the official fresh infusion, their employment is, strictly speaking, not absolutely justifiable when the fresh infusion is ordered, since alcohol is introduced into the medicine. This, however, is almost an academic point, and the actual quantity would be very small.

All concentrated infusions should be tested for salicylic acid, a preservative sometimes found, which is used to save the cost of alcohol. The liquid should be freed from alcohol by evaporation, acidified with H_2SO_4 , shaken with a mixture of equal volumes of ether and petroleum ether, the solvent separated and extracted with dilute aqueous potash solution, and this solution, containing the salicylic acid, neutralized and treated with a few drops of iron alum solution, when a purple colour results if salicylic acid be present.

Infusion of	Specific Gravity.	Extractive Gr. per 100 c.c.	Alcohol by vol.	Extraction of fresh Infusion $\times 8$.
Calumba . . .	0.985 to 0.995	2.5 to 3.5	15 to 20	3.2 to 3.5
Cascarilla . . .	0.980 „ 0.996	2.5 „ 4.6	15 „ 18.5	5.0 „ 6.0
Gentian (compound)	0.990 „ 1.010	6 „ 10	14 „ 19	8.5 „ 10
Quassia . . .	0.975 „ 0.985	0.1 „ 0.4	14 „ 20	0.2 „ 0.3
Rhubarb . . .	0.996 „ 1.010	9 „ 12.8	13 „ 18	12 „ 15
Senega . . .	1.000 „ 1.035	9 „ 12.5	15 „ 18	10 „ 14

THE SPIRITS OF THE PHARMACOPŒIA.

On opposite page are the characters which the various spirits of the Pharmacopœia should have.

The following method is recommended by Thorpe and Holmes (Proc. Chem. Soc. **19**, 13) for the determination of ordinary alcohol in essences and medicinal preparations containing essential oils and volatile substances, such as ether, chloroform, benzaldehyde, camphor, and compound ethers, in preparations for which "drawback" is claimed from the Inland Revenue, on exportation. It has been used for some time past in the Government Laboratory, and has been found to be both accurate and of very general applicability. Twenty-five c.c. of the sample, measured at 15.5°C . are mixed with water in a separator to a bulk of from 100 c.c. to 150 c.c. and common salt is added in sufficient quantity to saturate the liquid. The mixture is now shaken vigorously for five minutes with from 50 c.c. to 80 c.c. of light petroleum boiling below 60°C . and after standing for about half an hour the lower layer is drawn off into another separator, extracted if necessary a second time with petroleum, and then introduced into a distillation flask. Meanwhile, the petroleum layers are washed successively with 25 c.c. of saturated brine, the washings added to the main bulk, which is neutralized, if necessary, and then distilled, and the distillate made up to 100 c.c., and its relative density determined at the standard temperature in the usual manner. The results thus obtained require

a small correction from the circumstance that, as the alcohol present is distilled into four times its initial volume, the errors of the spirit tables are necessarily quadrupled. The mean error of the tables at

	Specific Gravity.	Optical Rotation in 100 mm. tube.	Other Characters.
Spirit of ether	0.801	nil	About 33 should distil below 45° C.
Compound spirit of ether .	0.808 to 0.812	nil	(see p. 485)
Spirit of nitrous ether .			(see p. 484)
Aromatic spirit of ammonia	0.838 ,, 0.845	nil	To contain 2.88 grms. NH ₃ in 100 c.c.
Fetid spirit of ammonia .			Alcohol 81 by vol. Aniseed oil 10 by vol.
Spirit of aniseed . . .	0.848 ,, 0.850	too small to observe	
Compound spirit of horse- radish	0.895 ,, 0.900	" "	
Spirit of cajaput . . .	0.842 ,, 0.844	" "	Alcohol 81 by vol. Cajaput oil 10 by vol.
Spirit of camphor . . .	0.848 ,, 0.851	+3.4° to +3.5°	Alcohol 81 by vol.
Spirit of chloroform . .	0.867 ,, 0.868		Alcohol 85.5 by vol.
Spirit of cinnamon . .	0.853 ,, 0.855	too small to observe	Alcohol 81 by vol. Oil 10 by vol.
Spirit of juniper . . .	0.836 ,, 0.837	0° to -0° 50'	Alcohol 81 by vol. Oil 10 by vol.
Spirit of lavender . . .	0.839 ,, 0.841	-0° 20' to -0° 55'	Alcohol 81 by vol. Oil 10 by vol.
Spirit of peppermint . .	0.840 ,, 0.843	about -2° 30'	Alcohol 81 by vol. Oil 10 by vol.
Spirit of nutmeg . . .	0.838 ,, 0.842	+1° 20' to +3°	Alcohol 81 by vol. Oil 10 by vol.
Spiritus rectificatus . .			(see p. 273)
Spirit of rosemary . . .	0.8405 ,, 0.8425	0° to +1°	Alcohol 81 by vol. Oil 10 by vol.
Spiritus vini gallici . .			(see brandy, p. 286)

below 40 per cent proof (for example, 0.972 sp. gr.) may be set down as +0.2 per cent of proof spirit, and hence the observed determinations, expressed as percentage, of proof spirit, require a subtractive correction of 0.8 per cent.

The essential oil, which should amount to 10 per cent by *volume* in the case of all the spirits of essential oils in the above table, may

be determined by evaporating the petroleum ether in a current of warm air and weighing the oil; or approximately by introducing 10 c.c. into a 200 c.c. flask with a neck graduated in $\frac{1}{16}$ c.c. and adding brine, shaking gently, and adding sufficient brine to drive the oil into the neck, where, after standing for twenty-four hours, it is measured.

CHAPTER IX.

DRUGS CONTAINING ALKALOIDS, ETC., CAPABLE OF APPROXIMATE DETERMINATION.

THE present chapter is devoted to a number of drugs containing physiologically active substances which are capable of determination with more or less accuracy, and are frequently of an alkaloidal nature. The principal active substances of this type often exist in very small amount in their respective drugs—sometimes to the extent of less than 1 per cent, but occasionally, as in opium, to a comparatively large extent. The difficulty of exact determination is considerably increased when more than one alkaloid exist together—and in some cases a separation is practically impossible.

The Nature of the Alkaloids.—The greater number of alkaloids are derivatives of cycloid bases such as pyridine and quinoline or of complex phenanthrene compounds. The majority of the alkaloids contain carbon, hydrogen, nitrogen, and oxygen, and are then generally crystalline solids. Those which are free from oxygen are usually liquid—such as conine. A small number are closely related to uric acid such as caffeine and theobromine but there is a tendency to restrict the name “alkaloid” to the derivatives of nitrogenous cyclic compounds. Generally speaking the alkaloids are bases analogous to ammonia, combining with free acids without the elimination of water.

Many of them are powerfully alkaline, neutralizing acids perfectly and forming well-defined crystalline salts. In some cases, however, their basic properties are very weak and even their salts with the mineral acids are decomposed by solution in water. The majority of the alkaloids are very sparingly soluble in water. Hence they are usually precipitated from solutions of their salts by alkalies. Nearly all the alkaloids are easily soluble in alcohol. Their salts are usually soluble in water and fairly so in alcohol. Numerous double salts exist which are practically insoluble in water, a fact which enables most of them to be precipitated in a highly insoluble condition.

The solvents which dissolve most of the alkaloids are amyl alcohol and chloroform, but many of them are freely soluble in other organic liquids. In most cases the salts are not soluble in these solvents. These facts are taken advantage of in the separation of the alkaloids from the crude drugs.

Numerous reagents have from time to time been proposed as precipitants of the alkaloids, of which the following are the most important.

Sonnenschein's Reagent.—This reagent, which is exceedingly useful for separating most of the alkaloids from foreign matters, consists of a solution of phosphomolybdic acid. It is best prepared from ammonium molybdate, by dissolving sodium phosphate in hot water, rendering acid with nitric acid, and adding an excess of a saturated solution of ammonium molybdate. The yellow precipitate formed is filtered off, washed, rendered acid with nitric acid and dissolved in warm solution of Na_2CO_3 . The solution is evaporated to dryness and ignited to drive off ammonium salts, and the cold residue again moistened with nitric acid and ignited. The product is phosphomolybdate of sodium. It is dissolved in ten times its weight of water containing 10 per cent of nitric acid and is then ready for use.

This reagent gives a yellow amorphous precipitate with nearly every alkaloid, but other substances than alkaloids are sometimes precipitated so that a precipitate does not always indicate the presence of an alkaloid; whilst a negative reaction is usually proof of the absence of alkaloids. To recover the alkaloid from the precipitate for further examination, the moist precipitate is treated with a solution of ammonia and the aqueous liquid, in which the alkaloid will often be floating as a white precipitate, is extracted by chloroform, amyl alcohol, etc. If a blue or green colour results it indicates reduction of the molybdic acid attended with decomposition of the alkaloid. If this is the case, the moist precipitate should be made into a paste with sodium carbonate and extracted with absolute alcohol.

Mayer's Reagent is a solution of potassio-mercuric iodide. It is a valuable precipitant of most alkaloids, and was originally recommended as a solution for the volumetric determination of alkaloids. The composition of the precipitate varies, however, with the slightest change in the conditions of the experiment, so that it has fallen into disrepute—so far as quantitative work is concerned. It is made by dissolving 6.775 grms of HgCl_2 in water, and 25 grms. of KI in an equal volume of water. The two solutions are mixed and the mixture made up to 1 litre. As a test for alkaloids it should be applied to solutions rendered faintly, but distinctly, acid with HCl or H_2SO_4 , and as free from alcohol as possible. Alkaloidal precipitates with Mayer's reagent are generally only faintly yellowish in colour and are amorphous and flocculent. They are soluble to some extent in alcohol, acetic acid and in much excess of the reagent. Other substances are precipitated besides alkaloids, so that an examination of the precipitate is necessary, especially when the solution is known to contain other organic matters. The quantitative determination of alkaloids by Mayer's solution is dependent on the fact that the solution above given is approximately one-twentieth normal: but it is necessary for anything like accuracy to check the value of the solution against a known quantity of the alkaloid being determined, since the conditions will vary with different alkaloids, and the composition of the precipitates is uncertain and variable. A. B. Lyons has examined the question very fully, and for further criticisms on it, the reader is

referred to his "Manual of Pharmaceutical Assaying". The following is the method of carrying out the titration. The liquid containing the alkaloid should be acidulated with hydrochloric acid, and adjusted as nearly as possible to contain about 0.5 per cent of alkaloid. Mayer's solution is run in carefully up to the point of no further precipitate being produced. There is no possible indicator for the end reaction, so that a few drops must be filtered through a very small filter and dropped on to black glass or ordinary glass backed with black paper. A drop of the solution from the burette is added, and the slightest turbidity can then be noted. All the trial portions must be returned to the titration flask before the final reading is decided upon.

Phosphotungstic Acid (Schleibler's reagent) is prepared by dissolving 100 parts of sodium tungstate and 60 parts of sodium phosphate in 500 parts of water, and adding sufficient nitric acid to produce an acid reaction. The general characters of this reagent are exactly the same as those of phosphomolybdic acid.

Wagner's Reagent is a 2 per cent solution of iodine in a 5 per cent potassium iodide solution. It should be added to solutions of the alkaloids rendered slightly acid with sulphuric acid, and only a small quantity is to be used—not sufficient to colour the liquid yellow. Under these circumstances a red or red-brown precipitate is produced in very dilute alkaloidal solutions. A negative reaction is practically proof of the absence of all common alkaloids, but a positive reaction requires confirmation. Much alcohol should be avoided, since the precipitation is often very slow in the presence of alcohol. The precipitates consist of complex iodides of the alkaloids, from which the free bases may be recovered by treatment with sulphurous acid or sodium thiosulphate, and then adding alkali and extracting with chloroform.

Dragendorff's Reagent is a reagent of great delicacy. It is a solution of potassio-iodide of bismuth, and is easily prepared by mixing 15 c.c. of liquor bismuthi of the Pharmacopœia, with 1 c.c. of HCl and then adding 1.2 grms. of potassium iodide. With this solution, solutions of the alkaloids strongly acidulated with H_2SO_4 give orange red precipitates which are quite insoluble in water.

Gold and Platinum Chlorides are useful precipitants of the majority of the alkaloids. They are best added as aqueous solutions to solutions of the alkaloid containing pure HCl. The melting-points of these double salts are generally characteristic and assist in the identification of the base.

Colour Reactions.—The minute quantities of the alkaloids with which one often has to deal, together with the fact that these bodies are so similar in their behaviour to most reagents, have caused very numerous colour reactions to be published. Colour reactions are, of course, the most unsatisfactory reactions with which the analyst has to deal, and it may be definitely stated that the great majority are totally useless, whilst many are only sufficiently reliable to give general indications. It is only a very few colour reactions that can be regarded as characteristic, and giving definite information.

Absolute purity of the reagent used is necessary. For example, the merest trace of nitric acid, a not uncommon impurity, in sulphuric acid will act as an oxidizing agent and may materially alter the colour of the reaction.

A careful examination of the mass of published work on colour reactions of the alkaloids compels one to reject the bulk of them as useless and misleading. Non-alkaloidal bodies will often give colours identical with well-known alkaloids, and several alkaloids will often give indistinguishable colours.

For instance, nitric acid is usually described as giving the following colours when a drop is placed on a fragment of the alkaloids:—

Codeine	= orange-yellow
Papaverine	= orange
Sabadilline	= yellow
Morphine	= yellow to red
Berberine	= red to red-brown
Brucine	= blood-red
Pseudomorphine	= orange-red

Again, Fröhde's reagent (5 mgs. of sulphomolybdic acid in 1 c.c. of H_2SO_4) is generally stated to give the following colours:—

Codeine	= deep blue (gradual)
Morphine	= violet-blue to dirty green and then to deep blue
Narcine	= yellowish-brown to red, to blue
Berberine	= brown-green
Quinine	= pale green
Apomorphine	= green to violet

And the same holds good for other reagents. Such few colour reactions (such as that for strychnine with chromic acid and H_2SO_4 ; or the thalleoquin reaction) as yield useful confirmatory information are mentioned under the alkaloids treated in the present chapter.

The Estimation of Alkaloids.—The alkaloids are rarely found in a free state in a plant, possibly never. They are combined with either an organic acid such as malic or tannic acid, or with an inorganic acid. The alkaloidal salt is usually, almost invariably indeed, soluble in alcohol, but not so in ether.

It is necessary in the first place to extract the alkaloid from the drug as free from foreign matter as possible, and then either purify it thoroughly and weigh it, or obtain it in as nearly a pure condition as possible and titrate it.

Allen ("Commercial Organic Analysis") gives the table on opposite page as showing the solubility of the leading plant constituents in water, alcohol and ether, thus indicating the bodies likely to be extracted from given plants.

As a rule the alkaloids can be best extracted from drugs by means of alcohol (90 per cent), (sometimes after the addition of an alkali) and then extracted from the alcoholic solution by the use of immiscible solvents. In some cases, however, it is better to render the drug alkaline so as to liberate the alkaloid, and extract with ether or chloroform at once, and then further purify the extracted alkaloid. Speaking generally, salts of alkaloids are insoluble in

solvents which are immiscible with water, hence an aqueous solution of an alkaloid rendered slightly acid and shaken with chloroform, ether, benzol, etc., does not lose its alkaloid. Glucosides, on the other hand, are dissolved out by the immiscible solvent. A certain number of alkaloids which possess very weak basic properties are, however, extracted from acid solution by immiscible solvents.

In shaking out an alkaloid by means of an immiscible solvent, the aqueous solution should be rendered alkaline, with ammonia or fixed

	Water.	Alcohol.	Ether.
Alkaloidal salts	Soluble	Soluble	Insoluble
Other salts of inorganic acids	Mostly soluble	Mostly insoluble	"
Other salts of organic acids	Soluble	Soluble	Mostly insoluble
Free organic acids	"	"	"
Tannin and colouring matter	"	"	Variable
Sugars	"	"	Insoluble
Gums and pectins	"	Insoluble	"
Albumenoids	"	"	"
Starch	" (in hot water)	"	"
Cellulose	Insoluble	"	"
Resins	"	Soluble	Variable
Fixed oils	"	Sparingly soluble	Soluble
Essential oils	"	Soluble	"
Chlorophyll	"	"	"

alkalis or alkaline carbonates. It is usually necessary to shake the aqueous liquid at least three times with fresh portions of the immiscible solvent to ensure complete extraction, especially when ether is used, as ether is very soluble in water and after separation of the two liquids, a considerable amount of ether, with alkaloid dissolved in it, remains dissolved in the water after the first extraction. The methods and solvents applicable to the various drugs described will be found in detail under each drug.

After the alkaloid is obtained in a more or less pure state—which may have required further extraction from the immiscible solvent by acidulated water and then a final extraction from this water after being rendered alkaline, with the immiscible solvent again—it may be weighed after evaporation of the solvent, and if it is of such a nature that it has been obtained in a state of purity, the weight may be taken as being that of the alkaloid, or, if it be impure, it may be determined by dissolving the residue, on evaporation of the solvent, in an excess of standard acid, usually one-twentieth normal, and titrating the excess of acid by standard solution of an alkali, baryta being preferable, although soda is generally used. Of indicators methyl-orange is the most generally useful, although rosolic acid, iodeosine, cochineal, and litmus frequently give good results.

When a complex or more or less unknown organic mixture is to be examined for alkaloids, it is best to precipitate any alkaloids that may be present from a solution containing but little alcohol, and a slight excess of hydrochloric acid, by one of the alkaloidal reagents

described above (p. 502). It is necessary to remove as much as possible of the inert organic matter present, which is best effected by a solution of lead acetate, which should be added to the *neutral* solution as long as any precipitate is formed. Much excess should be avoided. After the lead precipitate containing much organic matter has been filtered off and the precipitate washed with water, a little solution of basic acetate of lead is added which produces a further precipitate. The liquid is then rendered faintly alkaline with ammonia, again filtered, evaporated till the ammonia is driven off, and then the excess of lead removed by a current of sulphuretted hydrogen. After filtering and driving off excess of H_2S , the alkaloids are precipitated by one of the general precipitants such as phosphomolybdic acid, after the solution is rendered faintly acid with hydrochloric acid. The solution should stand for twelve hours and then be filtered. The precipitate is rendered alkaline with potassium carbonate and the free alkaloid is extracted therefrom with strong alcohol.

ACONITE.

The root of *Aconitum napellus* is official in the British Pharmacopœia, but no standards are given.

The potency of this drug is due to the presence of the alkaloid aconitine (see below), but there are also present small quantities of aconine $C_{25}H_{41}NO_9$ and picroaconitine. Good roots contain from 0.45 per cent to 1 per cent of alkaloids.

Aconite root contains from 3 per cent to 6 per cent of mineral matter, any excess over this amount indicating the presence of earthy matter.

The determination of alkaloids is carried out by exhausting the roots in fine powder with 75 per cent alcohol rendered slightly alkaline with ammonia. Twenty grms. of root are so treated and the resulting alcoholic liquid is evaporated to a syrupy consistence. This is then rendered alkaline with ammonia and extracted with three successive portions of 20 c.c. of chloroform. The chloroform solutions are extracted twice with dilute hydrochloric acid which is again rendered alkaline and the purified alkaloids finally extracted with chloroform three times, and the solvent evaporated. The weight is recorded, and the residue is then titrated by adding excess of one-twentieth normal acid and titrating back with one-twentieth normal alkali, using methyl-orange as indicator. One c.c. of one-twentieth normal acid is equivalent to 0.03225 gm. of aconitine (this determination is not quite exact, as aconine and picroaconitine, with different molecular weights, are present).

Tincture of Aconite.—This official preparation is an extract of 1 ounce of the drug with sufficient 70 per cent alcohol to make 1 pint. No official standards are given, but a genuine tincture should have the following characters:—

Specific gravity	0.890 to 0.895
Solid residue	1.3 „ 1.6 gr. per 100 c.c.
Alcohol by volume	66 „ 68 per cent
Alkaloids	0.025 „ 0.065 per cent

The alkaloids are determined by evaporating 100 c.c. to 200 c.c. of the tincture to a syrupy consistence, and then proceeding as above, weighing the alkaloids (by titrating them rather lower results will be obtained).

Aconitine is official in the Pharmacopœia, where its formula is wrongly given as $C_{33}H_{45}NO_{12}$. It is there described as melting at 189° to 190° , and when heated slightly above this temperature it yields acetic acid. It is soluble in 90 per cent alcohol, and chloroform, but less readily so in ether. It is nearly insoluble in water and petroleum spirit. It is lævorotatory. A drop of a solution of less than one-tenth per cent strength produces a persistent tingling sensation on the tongue. The salts are crystalline, the hydrochloride melts at 149° and the hydrobromide at 164° . A dilute solution—1 in 4000 in water—faintly acidulated with acetic acid yields a red crystalline precipitate with a few drops of a dilute solution of permanganate of potassium.

The true formula for aconitine is $C_{34}H_{47}NO_{11}$. It melts at 195° or if heated slowly at 182° , with decomposition. If 10 mgs. be evaporated with 3 c.c. of fuming HNO_3 and the residue on cooling be treated with KOH solution no violet colour is produced—(differentiation from atropine and pseudoaconitine); 0.1 grm. should require 3.3 c.c. of $\frac{N}{20}$ hydrochloric acid for neutralization, as determined by dissolving it in excess of the acid and titrating back with $\frac{N}{20}$ caustic soda solution using methyl orange as indicator.

The hydrochloride has the formula $C_{34}H_{47}NO_{11} \cdot HCl \cdot 3H_2O$; hydrobromide $2[C_{34}H_{47}NO_4 \cdot HBr]5H_2O$; and nitrate $C_{34}H_{47}NO_{11} \cdot HNO_3$.

ALOES.

The drug known by this name is the inspissated juice of the leaves of various species of aloe. There are numerous varieties of the drug, of which those known as Barbadoes aloes, Socotrine aloes, Cape aloes, and Natal aloes are the principal. The aloes official in the British Pharmacopœia are two in number. *Aloe Barbadosensis* is described as the product of *Aloe vera*, *Aloe chinensis* and probably other species. It occurs in yellowish, red-brown or almost black masses, with either a dull waxy, or smooth glassy fracture. The following are the official tests for this variety of aloes, which are known as either Barbadoes aloes or Curaçao aloes. The powdered drug imparts a crimson colour to nitric acid, and when treated with sulphuric acid and the vapour of nitric acid, should only yield a slight bluish-green colour, but not a bright blue colour (absence of Natal aloes). It is almost entirely soluble in a mixture of 2 volumes of 90 per cent alcohol with 1 volume of water. At least 70 per cent should dissolve in cold water.

Socotrine aloes is described as the product of *Aloe Perryi* and probably other species. It has always a dull waxy fracture, and is known in commerce as Socotrine or Zanzibar aloes. These aloes impart a reddish or yellowish-brown colour to nitric acid. If the

vapour of nitric acid be blown over the powder moistened with sulphuric acid, no blue coloration is produced. The Pharmacopœia also requires about 50 per cent to be soluble in cold water, and states that the drug is almost entirely soluble in a mixture of 2 volumes of 90 per cent alcohol and 1 volume of water.

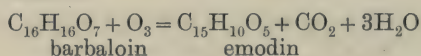
Natal and Cape aloes are not official in the British Pharmacopœia. The principal constituent of aloes is known as aloin. There are several varieties of "aloin" of which the following are the principal:—

Barbaloin $C_{16}H_{18}O_7$, $3H_2O$ (the Pharmacopœial formula is $C_{16}H_{16}O_7$), is a yellow crystalline powder sparingly soluble in cold water, insoluble in ether and fairly soluble in 90 per cent alcohol. It occurs in Barbados aloes, Curaçao aloes, Cape aloes, etc., in fact in every variety of aloes known, except Natal aloes, so that it might well be described as aloin without any prefix. It melts at 147° . It is usually found mixed with iso-barbaloin, although the best Barbadoes aloes contains about 20 per cent of barbaloin and practically no iso-barbaloin. Curaçao aloes contains about equal quantities of the two varieties. Léger considers barbaloin to have the formula $C_{21}H_{26}O_9$, but it is probable that its formula is $C_{16}H_{16}O_7$. Nataloin ($C_{23}H_{26}O_{10}$ Léger) and homonataloin ($C_{22}H_{24}O_{10}$, Léger) are found in Natal aloes.

Capaloin, another variety of aloin, is present in Cape aloes.

The neutral resinous matter of Barbadoes aloes is principally composed of the cinnamic ester of an alcohol, aloeresinotannol $C_{22}H_{26}O_6$, whilst that of Cape aloes is the paracumic acid ester of the same alcohol.

All varieties of aloes which contain barbaloin also contain a small amount (about 0.2 per cent) of emodin, a trioxy-methyl anthraquinone. It is probable that this is really the cathartic principle of aloes, since barbaloin is rapidly oxidized to emodin by the action of the air, if it be dissolved in alkalis:—



The Examination of Aloes.—The ash of genuine aloes varies from 1 per cent to 4 per cent, rarely exceeding 3 per cent. The water varies from 9 per cent to 14 per cent. The only other quantitative determinations available are the amounts soluble in water and alcohol (which should correspond with the above-given Pharmacopœial requirements); and an approximate determination of the aloin. Tschirch ("Pharm. Post." [37], 233, 149, 265) consider the following an accurate process:—

Since the active principles of all aloes, chiefly aloins, are soluble in $CHCl_3$, while the inert resins are insoluble, the determination of the $CHCl_3$ soluble constituents suffices for the assay. Five grms. of aloes are macerated for twelve hours with 5 c.c. of methyl alcohol, then warmed to $50^\circ C.$ to $60^\circ C.$ and treated with 30 c.c. of $CHCl_3$. After thorough agitation the mixture is set aside and the chloroform separated and filtered into a tared flask. The residue is again treated with another portion of $CHCl_3$, the solution added to that first obtained, the solvent distilled off, and the residue dried at

100° C., and weighed. Cape and Uganda aloes yield 80 per cent to 85 per cent of CHCl_3 extract, Socotrine aloes up to 55 per cent. The aloin in the chloroform residue may, if desired, be determined colorimetrically by Schouten's reaction, the production of a yellow colour and strong green fluorescence with a saturated solution of borax. A standard solution of 0.004 mgm. of aloin in borax solution is prepared; this shows a just visible green fluorescence when observed through a depth of 12 mm. in a vessel placed on black paper. A known weight of the above CHCl_3 residue is treated with a saturated aqueous solution of borax, and diluted until its degree of fluorescence is identical with that of the standard. A simple calculation then gives the amount of aloin present.

Tschirch and Hoffbauer ("Schenciz. Woch. für Chem. und Pharm." 42, 12) give the following as the average composition of commercial samples of aloes:—

Variety.	Aloin.	Other Chrysaminic Acid yielding bodies soluble in CH_2HO and CHCl_3	Substances not yielding Chrysaminic Acid soluble in CH_3HO and CHCl_3	Inert Resin.
	Per cent	Per cent	Per cent	Per cent
Cape aloes, soft	20	55	11.8	13.2
Cake aloes, hard	16	59	6.2	18.8
Uganda "	16	34	30.4	19.6
Barbadoes "	18	32	22.4	27.6
Curaçao "	18	32	16.6	33.4
Socotrine "	8	25	3.6	63.4

The Detection of and Distinction between Varieties of Aloes.—Léger gives the following reaction ("Jour. Pharm Chem." (6), 15, 335) for detecting aloes and for distinguishing between Cape and Barbados aloes.

Detection of Aloes.—0.05 grm. of the sample is dissolved in 100 c.c. of hot water. After rapid cooling in a current of cold water, the resin which is thrown down is filtered with the aid of a little talc; 20 c.c. of this filtrate are heated on a water bath to 80° C., when a few particles of sodium dioxide are added to the liquid. Simultaneously with the evolution of oxygen, the liquid becomes at first brown, then on adding more dioxide, a fine cherry-red colour.

Distinction of Cape and Barbadoes Aloes.—Twenty c.c. of the above filtrate are treated with 1 drop of saturated solution of cupric sulphate; the yellow colour is somewhat darkened; 1 grm. of NaCl is then added, the flocculent precipitate thus formed being disregarded, since it is re-dissolved in the 10 c.c. of 90 per cent alcohol, which is next added, Cape and Socotrine aloes give a vinous red colour which gradually fades to a permanent yellow tint. Barbadoes and Curaçao aloes give a bright cherry-red colour which persists for twelve hours. The first reaction is sensitive to a 1 per mille dilution of the aloes. Since the colour is then feeble, it may be rendered more evident by acidulating the coloured solution with HCl and shaking out with ether. The

etheral solution, when shaken with alkali, gives a marked cherry-red colour.

Detection of Aloes in Mixtures.—Since aloes are frequently prescribed associated with other drugs which contain oxy-methyl-anthraquinones, such as rhubarb, cascara, etc., these are best removed by the addition of a few drops of basic lead acetate solution. Aloins, in dilute solution, are only very slightly precipitated by this reagent, while the oxy-methyl-anthraquinones and their glucosides are completely thrown down. The above reactions are then applicable as described. Where only rhubarb is present with the aloes, alum and ammonia may be used as the precipitants, since rhubarb extract thus treated only gives the faintest peach tint with sodium dioxide. Incidentally it has been found that tinctures containing aloes which had been stored for several years failed to give the reaction with sodium dioxide, thus confirming the statement of Hirschsohn that these preparations are not stable.

Fawcett ("Pharm. Journ." [4], 19, 401) gives the following method for detecting the nature of aloes present in compound rhubarb pills. The coating of the pill is removed and the pill mass powdered. About 0.2 grm. of the powder is mixed thoroughly with 0.035 grm. of potassium ferricyanide. A small portion of the mixture is placed on a microscopic slide and made into a thin paste with water, and after it is spread out in a very thin layer, it is allowed to dry, and is then examined under the microscope, under a low power with lamplight. The following appearances will be presented:—

Socotrine.—Rounded pieces of a yellow colour (sometimes brown or green); often looking somewhat like potatoes.

Barbadoes.—Rounded pieces of a decidedly red colour, and similar in shape to Socotrine. This kind of aloes stains the ferricyanide red beyond the margin of the aloes itself.

Cape.—Irregularly shaped *glassy* pieces of a pale green colour.

If the ferricyanide at the edges of the spot is coloured even slightly red, either Barbadoes or Curaçao aloes is probably present. All the other ingredients of Pil. Rhei Co. appear to be unaffected by ferricyanide of potassium of the strength used.

The three kinds before named are probably those most likely to be used in pill-making at the present moment, but the following colour reactions with ferricyanide may be observed with some varieties of aloes not in such general demand viz.: Curaçao "Livery," greenish-brown; Curaçao, "Capey," greenish-brown, turning slowly crimson; Natal, pale greenish-brown; Zanzibar, pale brown.

If the still moist spots of the above experiments have the vapour of ammonia passed over them, "livery" Curaçao, Natal, Zanzibar, Socotrine and Cape all change to various shades of brown, but Barbadoes and "Capey" Curaçao turn purple.

Tschirch and Pedersen have examined the well-known test of Bornträger, which consists in extracting the sample with alcohol, filtering, shaking the residue left after evaporation of the alcohol with benzene, and shaking the benzene solution with ammonia, when a pink or violet-red colour is obtained. The colour takes some time to develop

and the liquids should be left standing for twenty-four hours. The substance to which this reaction is due is emodin. Most bodies which are derivatives of oxyanthraquinone yield this reaction, so that such drugs as araroba, rhubarb or cascara give the reaction. Practically all aloes except Natal aloes give the reaction.

Tschirch and Hoffbauer give the following details for the recognition of certain varieties of aloes:—

Recognition of Cape aloin.—A 0.1 per cent aqueous solution of the aloes gives a green fluorescence on the addition of 5 per cent of powdered borax.

Recognition of Aloe-emodin.—Ten c.c. of an aqueous 0.1 per cent solution of aloes is shaken for a minute with 10 c.c. of benzene. The separated benzene is withdrawn, and shaken with 5 c.c. of strong solution of ammonia. A rose colour is developed.

Distinction of Cape Aloes from Barbadoes Aloes.—Ten c.c. of a 0.1 per cent aqueous solution of aloes is treated with a drop of 5 per cent CuSO_4 solution. An intense yellow colour is developed by Cape aloes, which after the addition of a trace of NaCl and a little alcohol, does not change to red.

Distinction of Cape Aloes from Natal Aloes.—A spot of the yellow solution obtained by the action of strong H_2SO_4 on the aloes, placed in a porcelain capsule, should not develop a green colour with a trace of fuming nitric acid.

Anthraquinone Reaction of Aloes.—One grm. of the aloes is treated in a porcelain capsule with 20 c.c. of concentrated HNO_3 and heated on the water bath for two hours, the evaporated acid being made up from time to time; evaporation is then carried to dryness, and the residue, treated with water, leaves an insoluble brown powder. This dissolves in water containing ammonia, giving a violet-red colour.

Kremel ("Zeit. Analyt. Chem." xxxviii. 193) identifies aloes in medicinal combination by the following method, which gives excellent results:—

Solid substances are exhausted with alcohol, alcoholic or aqueous solutions are evaporated on the water bath, and the residues dissolved in alcohol or water respectively. The latter solution is again evaporated, and the residue taken up with water; the aqueous solution is then precipitated with excess of basic lead acetate, and the excess of lead removed from the filtrate by sodium sulphate. By these operations, all substances which interfere with the reactions are removed. The special reactions for aloin may then be applied. One of the most characteristic is the conversion into chrysammic and picric acids; the solution is evaporated to dryness, and the residue digested for some hours with 6 parts of concentrated nitric acid of specific gravity 1.45, 3 parts of water are added, and the solution heated on the water bath. On the further addition of water and cooling, the chrysammic acid separates in deep yellow to orange crystals. Chrysammic acid may be identified by the carmine-red colour of its alkali salts, the violet colour of its ammonium salt, and the insolubility of its barium salt. The picric acid is recognized by dyeing wool yellow.

Cripps and Dymond's method may be used to confirm the above

reactions when necessary. About 0.1 grm. of the substance if a solid, or of the solid residue if a liquid, is treated with 1 c.c. of strong H_2SO_4 in a porcelain dish and triturated until dissolved, three or four drops of strong nitric acid are then added and then 30 c.c. of water. A deep orange to crimson colour results according to the variety of aloes present. If ammonia be now added the colour is intensified, usually to a deep claret red. Senna and rhubarb are the only likely substances which interfere with this reaction.

Aloes in beer, in which it has occasionally been found as a hop substitute, is detected by extracting the beer with benzene, and applying the above reactions to the dry residue.

Aloin.—The aloin of the Pharmacopœia is barbaloin probably mixed with isobarbaloin. No good indication is given as to its preparation and no satisfactory tests are given. Pure barbaloin melts at 147° , but commercial samples are usually found to melt at about 140° to 153° and to contain from 1 per cent to 4.5 per cent of ash. Some samples contain resin, from which they should have been freed. If 1 grm. shaken up with 25 c.c. of warm distilled water yields a perfectly clear solution, it may be considered free from resin. One per cent or 2 per cent at most is the highest ash that should be obtained.

If a drop of CuSO_4 solution be added to an aqueous solution of aloin a bright yellow colour is produced, which is changed to red by the addition of a few drops of concentrated solution of sodium chloride, and to violet by the further addition of alcohol. If 1 grm. be shaken with 10 c.c. of petroleum ether and filtered, the filtrate should only give a faint pink colour when shaken with an equal volume of 5 per cent ammonia (limit of emodin).

Tincture of Aloes.—This official tincture is made by dissolving the solid extract of Barbadoes aloes and liquid extract of liquorice in 45 per cent alcohol. Genuine tincture of aloes has the following characters:—

Specific gravity	0.970 to 0.980
Solid residue	6.5 „ 7.6 per cent
Alcohol by volume	88 „ 42 „

Decoction of Aloes.—A comparatively weak decoction of aloes is official in the Pharmacopœia, and it is customary to use a concentrated decoction, using, of course, a proportionately less quantity in dispensing a prescription. Until recently, when proceedings under the Food and Drugs Act were taken, it was usual to use a concentrated decoction which could not possibly contain the proper amount of alcohol at so high a reputed degree of concentration. Most concentrated samples are now sold as three times the strength of the British Pharmacopœia, and the average decoction sold in retail shops is prepared by diluting this preparation with twice its volume of water. To correspond with the requirements of the British Pharmacopœia a genuine decoction should have the following characters:—

Specific gravity	1.004 to 1.006
Solid residue	5 „ 6.5 per cent
Alkalinity of ash as K_2CO_3	0.5 „ 0.6 „
Alcohol by volume	17 „ 18 „

Decoctions made by diluting concentrated decoctions whose reputed concentration is too high, will show a deficiency in alcohol and in solid residue; the latter must never fall below 5 per cent.

BELLADONNA ROOT.

This drug is official in the British Pharmacopœia, being the root of *Atropa Belladonna*. No official standard exists for the drug. When it is examined in the entire state, it is necessary to see that obviously foreign roots are absent, but the principal determination is that of the alkaloid present, as the drug is used to prepare the standardized preparations official in the British Pharmacopœia.

In the examination of the powder, the moisture should be determined, and the ash should not exceed 8 per cent.

A microscopic examination will also afford considerable information as to the purity of belladonna root.



FIG. 45.—Powdered belladonna root.

If any doubtful pieces of the whole root are noticed in a sample, transverse sections should be cut and compared with standard specimens. In the powder practically no sclerenchymatous cells are to be found, but plenty of parenchymatous cells, most of which contain starch grains which measure from 15 to 20 μ , with a few very

small ones, and a few measuring up to $30\ \mu$. There are plenty of vessels to be found and many elongated tracheids with narrow blunted points and numerous large round or oval pits, often arranged in a left ascending spiral.



FIG. 46.—Powdered belladonna leaves $\times 240$. *ccr*, cells with sandy crystals of calcium oxalate; *co*, collenchymatous cells from cortical tissue of midrib; *ei*, epidermis of under surface; *en*, epidermis over the veins, with striated cuticle; *es*, epidermis of upper surface, with striated cuticle and occasional stomata; *l*, bast; *me*, branching cells of spongy parenchyma; *nv*, fragments of small vein; *pa*, *p'a'*, palisade cells; *pg*, glandular hairs, long and short, with unicellular and pluricellular glands; *st*, stomata surrounded by three or four cells, one of which is smaller than the others; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, etc. (Greenish & Collin).

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The following analyses are those of E. M. Holmes, on air-dried specimens of the root:—

	Woody Roots.	Soft Roots.
	Per cent	Per cent
Moisture . . .	7.94	10.28
Soluble ash . . .	3.43	2.20
Insoluble ash . . .	4.60	3.68
Alcoholic extract . . .	22.53	29.87
Aqueous extract . . .	15.96	10.50

Determination of Alkaloids.—The official process for the determination of the alkaloids of belladonna root is described on page 517 under liquid extract of belladonna. The principal alkaloid present is hyoscyamine. The statement that atropine is present appears to lack confirmation. It is possibly formed during the extraction of the root, being isomeric with hyoscyamine. It is probable that scopolamine and traces of other alkaloids are present. These are described on pp. 520 and 521 (atropine, hyoscyamine and hyoscyne). Dunstan and Ransom have devised the following process for the assay of this root, and in the author's opinion it gives satisfactory results :—

Twenty grms. of the dry powdered root first moistened with a dilute solution of caustic soda are exhausted in a Soxhlet tube by a mixture of equal volumes of chloroform and absolute alcohol. The solution is removed and washed twice with 25 c.c. of water. If separation is not rapid the solution should be warmed. The chloroform retains nearly all the colouring matter, the alcohol and alkaloids as salts passing into the water. The watery liquid is washed with chloroform, and then rendered alkaline with ammonia, and extracted twice with chloroform. The alkaloids are dissolved out by the chloroform, which is then evaporated after being once washed with very dilute ammonia water. The residue is dried and weighed, or titrated in the manner described on page 506 with methyl-orange as indicator.

Keller's process ("Pharm. Post," 18, 67) as modified by Beckurts gives good results. Twenty grms. are dried and extracted with a mixture of 90 grms. ether and 30 grms. chloroform; 10 c.c. of a 10 per cent solution of caustic soda are then added and the whole shaken for three hours at intervals. Ten c.c. of water, or rather more if necessary to make the powder agglomerate, are then added, and the ether-chloroform separated. The aqueous liquid is washed twice with more ether, and the mixed solvents are extracted with 25 c.c. of centinormal hydrochloric acid. The acid is carefully separated, and the solvent washed with water, the washings being added to the first portion of acid liquid, and the acid titrated with centinormal alkali. From the amount of acid left, the amount of alkaloids can be calculated, each c.c. of centinormal acid consumed being calculated as equal to 0.00287 gm. of alkaloid.

F. C. J. Bird prefers the following method, which the author has used for some years and found very satisfactory :—

Belladonna root in fine powder	10 grms.
Potassium carbonate	2 grms.
Water	6 c.c.

Dissolve the potassium carbonate in the water, and rub the whole in a small mortar to a uniform moist granular powder.

Amyl alcohol	3 volumes	} g.s.
Chloroform	1 volume	
Ether	4 volumes	

Add the moistened powder to 20 c.c. of the above solvent, and macerate for half an hour, with occasional shaking. Force out the liquid by pressure and cover the powder with 10 c.c. more men-

struum. Agitate vigorously, let stand fifteen minutes and again force out the liquid. Repeat this at intervals of a quarter of an hour until six to ten quantities of menstruum have been used or the powder is exhausted.

Agitate the mixed ethereal liquids in a separator with—

Half saturated solution of chloride of sodium 10 c.c.

Run this off and reject. Rotate with 1 c.c. water, separate and shake the mixed ethereal extracts successively with—

Normal sulphuric acid	4 c.c. }
Water	6 c.c. }
Water	5 c.c. }
Water	5 c.c. }
Water	5 c.c. }

To the mixed acid solutions add—

Solution of ammonia *q.s.*

to render alkaline. Shake out the alkaloid with successive quantities of—

Chloroform	10 c.c.
Chloroform	10 c.c.
Chloroform	10 c.c.
Chloroform	5 c.c.

Run off the chloroform into a tared dish, evaporate, dry, weigh, and titrate as directed by the Pharmacopœia under liquid extract of belladonna. The figures obtained by weight and titration should not differ by more than 1 or 2 per cent.

Before passing on to the galenical preparations of the root, it will be convenient to briefly notice belladonna leaves, which are also official in the Pharmacopœia. These are the leaves of the same plant collected when the plant is in flower. No official tests exist.

Belladonna leaves should contain from 0.2 per cent to 0.6 per cent of alkaloids of which the principal part is hyoscyamine, the remainder being principally atropine.

The leaves may be assayed in the same manner as the root, but Dunstan and Ransom prefer to use boiling absolute alcohol to extract the powdered leaves with, diluting the alcoholic liquid with a large volume of dilute hydrochloric acid, and then removing fat and chlorophyll by means of chloroform. From the liquid thus purified, the alkaloids can easily be extracted by adding excess of ammonia and extracting with chloroform.

Liquid Extract of Belladonna.—This official preparation is an extract of the root, made with alcohol containing about 78 per cent of alcohol. It is to be of such strength that it contains 0.75 grms. of alkaloids in 100 c.c. No other standards are given officially.

A genuine extract should have a specific gravity of 0.890 to 0.920, and should contain from 11 per cent to 14 per cent of solid matter (but owing to the very variable strength of belladonna root, this figure is liable to vary outside these limits). The alcohol present in the finished extract should be from 66 to 69 per cent by volume.

The process by which this preparation should be assayed for official purposes is as follows:—

Ten c.c. are mixed in a separator with 10 c.c. of chloroform, 50 c.c. of water and a good excess of ammonia. After shaking and separating, the extraction with chloroform is twice repeated. The mixed chloroform liquids are washed with 15 c.c. of warm 5 per cent sulphuric acid twice. The mixed acid liquids are washed with 3 c.c. of chloroform, and then rendered alkaline with ammonia and three times washed with 10 c.c. of chloroform. The mixed chloroform liquids are washed with 5 c.c. of water containing 1 drop of ammonia, and the chloroform separated, and evaporated in a tared dish. The residue, dried below 100° C., is weighed and then dissolved in 10 c.c. of decinormal HCl. The excess of acid is determined by titration with centinormal soda, using cochineal as indicator. Each c.c. of centinormal alkali less than the 100 that would have been required for neutralization of the 10 c.c. of decinormal acid is equivalent to 0.00287 gm. of alkaloid.

As Bird has pointed out, the B.P. method consists of three operations: (1) The liberation of the alkaloid by ammonia and solution of the crude alkaloid in chloroform, (2) partial purification by conversion into sulphate, and (3) complete purification by again rendering alkaline with ammonia and shaking out the alkaloid with chloroform. The emulsification in stage (1) which has proved almost a complete bar to the use of this process as written, is well known, and some twelve months ago a modification was proposed (see "Pharm. Journ." [4], 8, 432) which consisted in first acidifying the diluted liquid extract, and then removing fatty and resinous bodies by agitation with chloroform, according to Dragendorff's plan, the acid chloroform being washed and the washings returned to the original liquid. Although this adds two more operations to the three already existing, infinitely less time is consumed in their performance; also the figures obtained are generally about 3 or 4 per cent higher, owing to there being less loss of alkaloid. Since that time an extended experience of this modified method has proved it to be an absolute preventative of the troublesome emulsifications incidental to the strict adherence to the process of the Pharmacopœia.

Tincture of Belladonna.—This preparation is official and is made by diluting 2 volumes of the liquid extract with 28 volumes of 60 per cent alcohol. A properly made tincture should have a specific gravity of 0.910 to 0.915 and should contain 0.6 gm. per 100 c.c. of extractive. Its alcohol strength is 57 to 58 per cent by volume. The only official standard, however, is that 100 c.c. when assayed by the process above described should contain 0.048 to 0.052 gm. of alkaloids in 100 c.c.

These preparations can, of course, be assayed by the other processes above described. If this is done, it is best to evaporate the bulk of the alcohol and then to commence the extraction of the more or less syrupy liquid.

Liniment of Belladonna.—This is also an official preparation of the root. It is a mixture of 10 ounces (fluid) of the liquid extract, with 1 ounce of camphor, 2 ounces of water, and sufficient 90 per cent alcohol

to produce 20 ounces. No official standards are given but the pure preparation should contain 0.375 grm. of alkaloids when assayed by the official process. In carrying out the process it is necessary first to dilute 20 c.c. of the liniment with very dilute sulphuric acid, and filter off the camphor precipitated, or the camphor may be removed by evaporation. Any camphor remaining is removed by the chloroform. A properly prepared sample should have a specific gravity of 0.875 to 0.890, and should contain about 6 per cent of non-volatile residue. Its alcoholic strength should be 70 per cent to 73 per cent.

There are two semi-solid extracts of belladonna official in the Pharmacopœia. The *alcoholic extract of belladonna* is made by evaporating the liquid extract to a syrupy consistency, and diluting with sugar of milk so that 20 fluid ounces of the liquid extract shall yield 15 ounces of semi-solid extract. This extract contains 1 per cent of alkaloids.

The *green extract of belladonna* is the juice of the fresh leaves and young branches of the plant, evaporated, with the removal of certain inert coagulable constituents. No standards are given.

In reference to these extracts, it has been pointed out by Farr and Wright ("Pharm. Journ." 4, 20, 546) that by an oversight the characters of the alcoholic extract are not given in the Pharmacopœia, and that the preparation is often sent out in the semi-solid form, whereas a powdered extract was intended. The green extract of the leaves contains a very variable amount of alkaloid, and as no standard exists officially, this preparation must be looked upon as an unsatisfactory one.

The following figures have been recorded:—

	Per cent
Barclay	0.77 to 1.24
Farr and Wright	0.52 „ 1.33
Naylor and Bryant	0.55 „ 1.80
Umney	0.94 „ 1.26

In determining the amount of alkaloids present in these solid or semi-solid extracts, the alcoholic extract must be thoroughly well mixed with water slightly acidulated with sulphuric acid, and the assay carried out on the liquid so obtained. For the assay of the green extract the process devised by Naylor and Bryant gives the best results. It is carried out as follows:—

From 2 grms. to 5 grms. of the extract are weighed into a wide-mouthed flask (for convenience an Erlenmeyer flask is recommended), 25 c.c. of 90 per cent alcohol is added, and the flask with its contents heated on a water bath under an inverted condenser or other arrangement that prevents loss of alcohol and provides facilities for exhaustion. This operation is twice repeated with two more quantities of 25 c.c. of 90 per cent alcohol. After each operation the alcoholic solution in the flask is allowed to become cold, and filtered, and the filtrates are united.

To make sure that extraction of the alkaloidal content is complete, the residue in the flask is warmed with a 5 per cent solution of hydrochloric acid and filtered. The filtrate is then tested with solution

of iodine in potassium iodide. Three extractions with alcohol are usually sufficient for the purpose.

To the alcoholic solution of the alkaloid an equal volume (75 c.c.) of a 5 per cent solution of the hydrochloric acid of the Pharmacopœia is added, and the mixture shaken up three times successively with 15 c.c. chloroform. After separation and rejection of the chloroformic liquids the acid solution is rendered distinctly alkaline by the addition of solution of ammonia and again shaken up three times successively with 10 c.c. chloroform. The chloroformic solutions, after separation are mixed and evaporated, and the residue dried over a water bath until it ceases to lose weight. The dry alkaloidal residue is titrated as the Pharmacopœia directs in the final stage of the process for determining the proportion of alkaloid as given under *extractum belladonnæ liquidum*.

The chloroformic separations take place quicker and cleaner than is the case in the Pharmacopœia process for liquid extract of belladonna.

It may be noted that the difference between the amount of alkaloid obtained by weighing and that indicated by subsequent titration is less than 0.01 gm.

Belladonna Ointment.—This official preparation should contain 0.6 per cent of alkaloids. No official method of assay is given. Bird has given the following process, which works well:—

Belladonna ointment, B.P.	10 grms.
Benzol	20 c.c.
Water	10 c.c.
Diluted sulphuric acid	7 c.c.

Melt the ointment in a small dish, pour into a separator, rinse the dish with the water and acid, add the benzol and agitate vigorously.

Separate the benzol, and wash the aqueous layer twice with successive quantities of

Benzol	10 and 10 c.c.
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Warm the mixed benzol washings with

Diluted sulphuric acid	3 c.c.
Water	3 c.c.

agitate and separate. Shake a second and third time with

Water	10 and 10 c.c.
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Reject the benzol and return the acid liquids to the separator. Then make alkaline with

Solution of ammonia	
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Shake out with three successive quantities of

Chloroform	10, 10, and 10 c.c.
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adding if necessary

Saturated solution of ammonium carbonate	q.s.
--	------

Wash the mixed chloroform solutions with

Water	3 c.c.
Solution of ammonia	10 drops.
Solution of ammonium carbonate	2 c.c.

Evaporate in a tared dish, dry below 100° C., weigh and titrate as directed in the Pharmacopœia.

Belladonna Plaster.—An official belladonna plaster exists which is a mixture of resin plaster (which contains colophony, soap and lead plaster) with liquid extract of belladonna from which the bulk of the liquid constituents have been removed by evaporation.

It should contain 0.5 per cent of alkaloids. According to Hender-son the best means of getting this preparation into a suitable condition for assay, is to disintegrate it with ether, and then shake the emulsion with a mixture of acetic acid and water, the alkaloids and lead being dissolved in the acid. The details of the method are as follows:—

Weigh 5 grms. of the plaster, and introduce it into a stoppered glass separator, with 25 c.c. of ether; allow the plaster to disintegrate. When the contents of the separator present the appearance of an emulsion, add 5 c.c. of a mixture of glacial acetic acid and water (three parts of the former to two parts of the latter), shake for thirty seconds and set aside until the acid liquor has completely separated. Draw off the lower layer into a small beaker, and again agitate the ether solution with 5 c.c. of the dilute acetic acid of the B.P. and draw off as before. To the united acid liquors in the beaker add dilute sulphuric acid in slight excess, stir well, and allow the sulphate of lead to subside. Filter the solution through a small filter into a separator, transferring the whole of the sulphate of lead on to the filter by means of a glass rod tipped with rubber; allow to drain. Remove the funnel from the separator, and wash the lead precipitate with distilled water until a drop of the filtrate gives no precipitate with Mayer's reagent. Concentrate the washings to a small bulk and add them to the contents of the separator.

It will be found to be advantageous to use a filter pump in washing the lead precipitate, but it is not essential. The separator now contains the extract of belladonna, freed from the other constituents of the plaster. Add excess of solution of ammonia and 10 c.c. of chloroform, shake well for thirty seconds, and draw off the chloroform into another separator. Repeat this treatment with two more successive portions of chloroform of 5 c.c. each. Mix the chloroformic solutions of the alkaloids, and shake out the alkaloids with three successive portions of dilute hydrochloric acid, using 5 c.c. for each shaking. To the mixed acid solutions, in a separator, add excess of solution of ammonia and 10 c.c. of chloroform, shake well, and draw off the chloroform into a weighed dish, repeat the shaking with two successive portions of chloroform, using 5 c.c. for each, draw off as before, and allow the chloroformic solutions to evaporate spontaneously. Dry the residue in the air oven at a temperature not exceeding 93° C., until the weight is constant, and weigh.

Atropine.—Atropine $C_{17}H_{23}NO_3$, and its sulphate $(C_{17}H_{23}NO_3)_2 \cdot H_2SO_4$ are official in the Pharmacopœia.

Atropine is described as being soluble in 300 parts of water (temperature not stated), and readily soluble in alcohol, chloroform, and ether. It has an alkaline reaction, and when applied to the eye has

a powerfully dilating action on the pupil. An alcoholic solution, on warming, precipitates a solution of mercuric chloride, the precipitate being yellow but soon turning red. The aqueous solution when treated with solution of auric chloride gives a citron yellow precipitate which when recrystallized from boiling water acidulated with HCl has a minutely crystalline appearance, and when dry a dull powdery appearance (distinction from hyoscyamine). When moistened with fuming nitric acid and evaporated to dryness on a water bath, the residue gives with freshly prepared alcoholic solution of potash, a fugitive reddish-violet colour. It leaves no ash.

Sulphate of atropine is a crystalline substance melting at 183°C .

It is insoluble in ether and chloroform, and leaves no ash on ignition.

No other official requirements are given.

As has been mentioned above, it is doubtful whether atropine exists in belladonna, and it is possible that it is formed by isomerization of the hyoscyamine present. It is frequently obtained from the rhizome of *Scopola Carniolica*. It is soluble in 500 parts of cold water, not 300 parts as stated in the Pharmacopœia, and should melt at 115° to 116° . Commercial atropine frequently contains a little hyoscyamine, which lowers its melting-point, but raises that of the aurichloride, which with pure atropine melts at 137° , as against 160° for the hyoscyamine compound. A solution of atropine is optically inactive, whilst that of hyoscyamine is optically active.

According to the British Pharmaceutical Codex, pure atropine sulphate melts at 185° to 186° .

Hyoscyamine.—The pure alkaloid, $\text{C}_{17}\text{H}_{23}\text{NO}_3$, which is isomeric with atropine is rarely employed. The only salt that is official is the sulphate $2(\text{C}_{17}\text{H}_{23}\text{NO}_3)\text{H}_2\text{SO}_4 \cdot 2\text{H}_2\text{O}$. This is described in the Pharmacopœia as a crystalline powder, deliquescent, odourless and having a bitter taste. It melts at 206° . It is soluble in 0.5 part of water and in 2.5 parts of 90 per cent alcohol. A solution in water yields no precipitate with platinum chloride, and with auric chloride it yields a precipitate of a yellow colour, soluble in boiling water acidulated with HCl, and deposited on cooling in the form of brilliant golden scales (distinction from atropine). It leaves no ash. According to the British Pharmaceutical Codex it is soluble in 4.5 volumes of 90 per cent alcohol. Commercial samples melt below 206° , but they should not be allowed to melt below 200° , or the limits of impurities will be too great. The free base crystallizes in needles or prisms.

If 10 mgs. be added to 2.5 c.c. of nitric acid, evaporated to dryness, and alcoholic solution of potash added, a violet colour should result.

Hyoscine.—This alkaloid, also known as scopolamine, is official in the form of its hydrobromide. It is described here for convenience, although it probably does not occur in belladonna root, but is obtained from other solanaceous plants. It is a mixture of stereo-isomeric varieties of the base, having the formula $\text{C}_{17}\text{H}_{22}\text{NO}_4\text{HBr} \cdot 3\text{H}_2\text{O}$ (hydrobromide). The Pharmacopœial formula is incorrect. The Pharmacopœia requires this salt to lose rather more than 12 per cent

of its weight on heating to 100° C. and the resulting mass to melt at 193° to 194°. It forms with auric chloride a compound melting at 190°. Its aqueous solution slightly reddens litmus. The statements as to its melting-point are rather misleading. When heated in a capillary tube the hydrated salt melts at about 100°. If dehydrated over sulphuric acid, the salt as met with in commerce melts at 181°. The purer optically inactive variety melts at 180°, and the levorotatory variety at 193°. The aurochloride formed without the addition of free hydrochloric acid melts at 215°, but the product formed in the presence of free HCl melts at 193°. The nitric acid colour reaction yielded by hyoscyamine is also yielded by hyoscyne. The free base is a syrup, and does not crystallize.

CANTHARIDES.

Cantharides, or Spanish flies, as the insects are often termed, are the dried beetle, *Cantharis vesicatoria*. This is the only variety which is official in the British Pharmacopœia under the name Cantharis. The "Chinese cantharides" or Chinese flies, are the dried beetle, *Mylabris cichorii*, and are quite similar in properties to the Spanish beetle, both of them being used as vesicating agents.

The important constituent of these beetles is cantharidin, which occurs both in the free and in the combined condition. The estimation of this ingredient is the most important determination to be made in their examination. The two varieties of cantharides have the following characters:—

	"Spanish flies,"	"Chinese flies."
	Per cent	Per cent
Mineral matter	5 to 6·5	4·0 to 5·8
Free cantharidin	0·3 " 0·57	0·6 " 1
Combined cantharidin	0·05 " 0·3	0·1 " 0·8
Total cantharidin	0·4 " 0·85	0·7 " 1·9
Moisture	10 " 13	10 " 13

The Chinese insects contain more cantharidin than the Spanish, and are the better source for the preparation of cantharidin.

Colledge ("Pharm. Journ." 1910, 674) has examined six samples of Cantharides of different species in the following manner:—

The powdered flies were exhausted with benzene and the solvent evaporated. The residue was exhausted with water slightly acidified with HCl, at boiling temperature. The acid solution was then exhausted with chloroform and the chloroform evaporated. This residue was then extracted with petroleum ether to remove fat, and the residue finally dried at 60° to 65°. The following amounts of cantharidin were obtained:—

Mylabris oculata	0.615 per cent.
„ holocericea	1.3 „
Decatoma lunata	1.0 „
Electica wahlbergia	0.32 „
Cantharis vellata	2.73 „
Lytta cœlestina	1.89 „

Chinese cantharides gave 1.2 per cent of cantharidin.

A useful method for the determination of cantharidin is that of Greenish and Wilson ("Pharm. Journ," 4, vi. 255). Their method is as follows:—

Determination of Total Cantharidin.—Twenty grms. of the flies in No. 40 powder are mixed in a small mortar with 25 c.c. of a mixture of:—

Glacial acetic acid	1 volume
Rectified spirit	2 volumes
Chloroform	3 „

The moistened mass is covered over for about an hour, and then allowed to dry spontaneously or at a *slightly* raised temperature. This is easily accomplished without loss of cantharidin. The dried mass is then packed in a Soxhlet extractor, and exhausted with chloroform, the latter being first used to rinse out the mortar employed.

About one hour will usually suffice for complete extraction, but complete exhaustion should always be ascertained by removing the flask with the chloroformic solution, and continuing the extraction with a little fresh chloroform.

The chloroformic solution thus obtained is placed in a separator containing a little water, and the acetic acid, which passes into the water, is almost, but not quite, neutralized with solution of potash, and the whole well shaken.

The chloroformic layer is run off into a glass dish and evaporated, cautiously towards the end. The residue consists of fat, in which can be seen crystals of cantharidin. The fat is removed by washing with petroleum spirit (the washings being set aside), leaving in the dish crystals of cantharidin mixed with a green substance of a resinous nature. This residue is allowed to dry, and is then washed with successive small quantities of absolute alcohol until all green matter is removed, and perfectly white cantharidin remains. The alcoholic washings are carefully evaporated.

The cantharidin, dissolved or mechanically removed whilst washing out the fat with petroleum spirit, is now recovered; 20 c.c. of 10 per cent solution of caustic potash are added to the petroleum spirit solution, and the mixture warmed until the fat is completely saponified; during the process most of the petroleum spirit is dissipated. The soap solution thus produced is diluted with warm water and transferred to a separator, sufficient petroleum spirit being added to dissolve the fatty acids when liberated; it is now acidified with hydrochloric acid, when the fatty acids rapidly rise and dissolve in the petroleum spirit. The aqueous layer is quickly run off from beneath the petroleum spirit solution into another separator, the petroleum

spirit solution washed with water and the washings added. The cantharidin is then removed by shaking with successive quantities of chloroform as long as cantharidin is removed; this must be ascertained. In the chloroformic solution thus obtained the residue from the alcoholic washings of the crystallized cantharidin is dissolved.

The chloroform now contains in solution chiefly cantharidin and the green resinous matter. It is placed in a separator and shaken with lime water, containing excess of calcium hydrate suspended in it, and solution of common salt, the latter causing the chloroformic layer to separate more readily.

In this way the cantharidin passes into aqueous solution, probably as cantharidate of calcium, whilst the chloroformic layer containing green resin and colouring matter is rejected.

The aqueous solution is filtered, acidified with hydrochloric acid, and shaken out with chloroform as before. This chloroformic solution is added to the cantharidin previously separated, evaporated cautiously, dried in a desiccator, and weighed. In this way a crystalline residue of cantharidin only very slightly coloured is obtained.

Determination of Free Cantharidin.—This is accomplished in the same way as the determination of total cantharidin, with the exception that the drug is not moistened with the acetic acid mixture before extraction, and, no acetic acid being present, the washing of the chloroformic solution with water becomes unnecessary.

A useful summary of the proposed methods for the assay of cantharidin by Self and Greenish ("Pharm. Journ." [4], 24, 324) has been published and the authors finally adopt the following process:—

Twenty grms. of cantharides in fine powder are moistened with 3 c.c. of strong HCl and extracted in a Soxhlet with 80 c.c. of benzene. The benzene is driven off, the last traces being removed in a current of air on a water bath. The benzene (recovered by distillation) is extracted three times with a 1 per cent solution of KOH to recover traces of cantharidin. The alkaline liquid is acidified with HCl, made up to 105 c.c. with water and added to the mixed fat and cantharidin in the extraction flask. The mixture is boiled for ten minutes under a reflux condenser, the fat allowed to separate and as much as possible of the aqueous solution transferred to a large separator. The boiling with water (50 c.c.) is repeated four times and the mixed aqueous extracts are rendered thoroughly acid with 3 c.c. HCl, and extracted four times with chloroform. The residue from the chloroform extract is washed three times with three portions of 5 c.c., 5 c.c., and 2 c.c. of equal volumes of absolute alcohol and petroleum ether which has previously been saturated with cantharidin. The washing fluid is poured through a funnel containing a plug of cotton wool, and the flask and wool finally washed with a few c.c. of petroleum ether, until nothing further is dissolved. A few c.c. of chloroform are then poured through the wool into the flask, in case any crystals of cantharidin have been transferred to the wool and the cantharidin in the flask dried to constant weight at 60° to 65°.

The following is the official process in the German Pharmacopœia:—

The powder is extracted with chloroform and dilute hydrochloric

acid for twenty-four hours, and an aliquot part filtered out and evaporated; the residue is extracted with petroleum benzin for twelve hours, the liquid filtered off, and the extraction repeated several times. The undissolved portion is then treated with water containing a trace of ammonium carbonate as long as this removes any colour, then dried and weighed; if it is then resinous or dark in colour, the cantharidin is extracted from it with hot acetone. Not less than 0·8 per cent of crystalline cantharidin is required.

The method proposed by Léger ("Journ. Pharm. Chem." 6, 17, 457) gives good results. He prefers to extract with benzene, after acidifying with hydrochloric acid.

Tincture of cantharides is an extract of 1 part of cantharides with 80 parts of 90 per cent alcohol. It is so weak in cantharidin as to be almost impossible to assay accurately. The following are the characters that a genuine tincture should have:—

Specific gravity at 15°	0·835 to 0·840
Residue dried carefully at 90° to 100°	0·15 gm. to 0·17 gm. per 100 c.c.
Alcohol	89 per cent to 90 per cent by volume.

CINCHONA.

There are a number of species of *Cinchona* whose barks are more or less rich in alkaloids of which quinine is the principal. Various species of *Remijia* also contain the same alkaloids.

Any of these plants may be used as the source of preparation of the cinchona alkaloids, but there is only one that is official for other purposes in the British Pharmacopœia. This is the bark of *Cinchona succirubra*, from which the galenical preparations of Cinchona should be made.

The Pharmacopœia requires that this bark, when used for any purpose other than that of obtaining the alkaloids and their salts, should contain from 5 per cent to 6 per cent of alkaloids of which not less than half should be quinine and cinchonidine when estimated by the following official method:—

Twenty grams of the bark in powder are mixed with 6 grms. of calcium hydroxide and moistened with water (20 c.c.); allow the mixture to stand for an hour or two, and then transfer the whole to a flask connected with a reflux condenser. Add 130 c.c. of a mixture of 3 volumes of benzol and one of amyl-alcohol, and boil for half an hour. Decant the liquid and repeat the boiling, etc., repeat a third time and mix the liquids and wash the powder on a filter with more of the liquid until exhausted. Place the mixed liquids whilst still warm in a stoppered separator. Add 2 c.c. of dilute HCl mixed with 12 c.c. of water. Shake well, and separate the acid layer. Repeat the extraction with slightly acidified water until all the alkaloids are extracted. Neutralize with ammonia, and concentrate to 16 c.c. Add about 1·5 grms. of sodium potassium tartrate dissolved in 3 c.c. of water, and stir the mixture with a glass rod. Insoluble tartrates of quinine and cinchonidine will separate in about an hour. These are collected on a filter, dried and weighed. They will contain 0·8 of

their weight of alkaloids, from which the percentage is calculated. To the mother liquor add ammonia in slight excess. Collect, wash, and dry the precipitate, which contains the other alkaloids. The sum of the two may be taken as the total alkaloids.

The principal alkaloids occurring in cinchona bark are the following. Of course, there are numerous other less important alkaloids, and they do not all occur associated in each species of cinchona, but those now described are the only ones having any practical importance from the analyst's point of view.

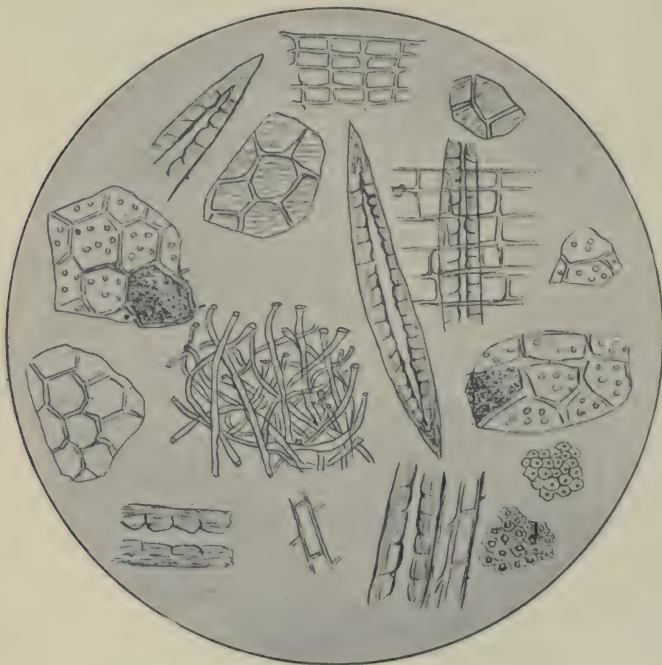


FIG. 47.—Powdered cinchona bark.

Quinine, quinidine, and quinicine are isomeric bases of the formula $C_{20}H_{24}N_2O_2$.

Cinchonine and cinchonidine are isomers of the formula $C_{19}H_{22}N_2O$.

Hydrocinchonine and hydrocinchonidine are isomeric, and have the formula $C_{19}H_{24}N_2O$.

Quinamine has the formula $C_{10}H_{24}N_2O_2$.

Cupreine has the formula $C_{19}H_{22}N_2O_2$.

Of these the only official alkaloid is quinine. This is described on p. 532 and tests for cinchonine and cinchonidine, which have some practical importance, will be found mentioned on the same page.

The examination of cinchona bark is confined to the determination

of the mineral matter: a microscopic examination (if in powder): and a determination of the alkaloidal value. If the last named be required for official purposes, the process described above should be used; but from the point of view of the manufacturer of the alkaloids, a fuller separation will be necessary.

The ash of cinchona bark should not exceed 4 per cent to 5 per cent.

Under the microscope the powdered bark should show very large bast fibre with characteristic pits, but no other cells should be found of a sclerenchymatous character. The parenchymatous cells are deep red or brown in colour, and after digestion in weak potash solution, crystals of precipitated alkaloids may be found.

As alternative methods for the assay of cinchona bark, the following are those which give the best results:—

De Vrij (modified).—Twenty grms. of the bark are powdered and mixed with 5 grms. of quicklime and 50 c.c. of water. The whole is dried at 70°, and transferred to a flask with a reflux condenser, and boiled with 200 c.c. of alcohol of at least 93 per cent strength. After an hour's boiling the liquid is cooled and filtered off. The residue is again boiled with 100 c.c. of the alcohol, and this is also filtered off. The residue is washed twice with 50 c.c. of alcohol, and the mixed alcoholic liquids are rendered acid with dilute sulphuric acid. Calcium sulphate is filtered off, washed with a little alcohol which is added to the main alcoholic liquid, and this is then concentrated to expel alcohol and again filtered, the insoluble matter being well washed with water acidulated with sulphuric acid. The filtrate which contains the alkaloids as acid sulphates is then transferred to a separator after being concentrated to about 40 c.c. and rendered alkaline by soda solution. The liquid is now extracted four times with 30 c.c. to 35 c.c. of chloroform, and the mixed chloroformic liquids, after being once washed with water, are evaporated, and the residue dried and weighed. The weight represents the total alkaloids in the 20 grms. of bark.

Prollius' Process (modified).—Ten grms. to 20 grms. of very finely powdered bark are treated with twenty times its weight of a mixture of 85 parts of ether, 10 of alcohol and 5 of ammonia of specific gravity 0.960 (all by weight).

Place the mixture in a well-fitting glass-stoppered bottle, weigh and shake well at intervals for four hours. Maintain the original weight by the addition, if necessary, of more solvent. Pour off as much as possible perfectly clear, rapidly closing the bottle again. Ascertain how much has been poured off by weighing the bottle again. Distil off the solvent, evaporate the residual liquid, and weigh the residue in a tared beaker, when dry. The weight of the residue represents the alkaloids in the portion of the solvent evaporated, and as the proportion of this to the whole amount used is known the percentage can be calculated.

Squibb's Method.—Squibb effects the exhaustion of the powdered bark by means of acetic acid of 10 per cent strength in an apparatus which is in effect a small percolator. The complete extraction of 10

grms. of the powder is effected in thirty-six hours, the entire percolate measuring 180 c.c. to 200 c.c. This percolate is evaporated until the residue, though still liquid whilst hot, is semi-solid on cooling. The weight of this residue usually amounts to about 35 per cent to 38 per cent of the bark used. The extract is dissolved in a mixture of ammonia and alcohol, more ammonia is added to ensure the liberation of the whole of the alkaloids, and the separation effected by shaking out with chloroform. The alkaloids are then taken up with decinormal sulphuric acid, precipitated with decinormal potassium hydrate, and again taken up with ether. Finally, the varnish-like residue left on evaporating the ethereal solution is weighed in order to get the approximate percentage of alkaloids, after which the alkaloids are titrated with decinormal acid.

Where a separation of certain of the alkaloids is required, the following processes may be employed. It is to be remembered, however, that nearly every process for the separation of cinchona alkaloids is only approximate, and will often give erratic results in unskilled hands.

As a rule the only practical question for solution is the amount of quinine that can be obtained from the bark, the remainder of the alkaloids being of but quite secondary importance.

For this purpose a modification of a process devised by De Vrij is fairly accurate if carefully carried out.

The alkaloids from 50 grms. of the bark, extracted by one of the above-described processes and in a fine state of division, are treated in a closed vessel with ten times their weight of pure ether free from alcohol. The mixture is well shaken and left for twelve hours, when it is filtered and the residue washed with a small quantity of ether. The ethereal solution is evaporated to dryness, and the residue weighed. It consists of quinine, quinidine, and cinchonidine in heavy traces, and amorphous alkaloids. It is dissolved in ten times its weight of 60 per cent alcohol and rendered acid with decinormal sulphuric acid. An alcoholic solution of iodine is then added until no further precipitation takes place. Excess of iodine must be avoided. A black precipitate of herepathite, an iodine compound of quinine of the formula $4C_{20}H_{24}N_2O_2 \cdot 3H_2SO_4 \cdot 2HI \cdot I + 3H_2O$, is formed. This is allowed to stand for twelve hours, and the precipitate is then filtered off, washed with strong alcohol, dried at 100° , and weighed. The weight multiplied by 0.55055 gives the weight of quinine in the mixed alkaloids operated upon.

De Vrij has later recommended using a solution of the iodosulphate of the amorphous mixture of alkaloids (known as quinoidine) as a precipitant instead of iodine. This prevents the possibility of the formation of periodized products.

David Howard, instead of converting the ethereal solution of quinine and impurities into this iodine compound prefers to agitate the ether with excess of dilute sulphuric acid, and, after heating the aqueous liquid to boiling, to add ammonia until the liquid is neutral to litmus, when the quinine crystallizes out almost entirely as sulphate (this salt is practically insoluble in a solution containing

ammonium sulphate). The crystals are filtered off, and washed with a little cold water, pressed between filter paper, and dried at 100°; 84.7 of the anhydrous salt are equivalent to 100 of the crystallized sulphate.

The summary on pages 530, 531 of De Vrij's process for the complete separation of the cinchona alkaloids is due to A. H. Allen.

Vigneron's Process.—This process ("Jour. Pharm. Chem." **21**, 180) gives excellent results if carefully carried out.

The total alkaloids of 25 grms. of bark are treated with twenty times their weight of pure ether and shaken well with five or six small pieces of pumice stone the size of a pea, previously moistened with 98 per cent alcohol. The small amount of alcohol thus introduced facilitates the separation of the quinine from the other alkaloids. The mixture is allowed to macerate for six hours at about 15° C., with occasional agitation, then filtered into a porcelain capsule, from which the ether is allowed to evaporate spontaneously. The residual alkaloids insoluble in ether are again macerated with a similar quantity of ether for twelve hours; the ethereal liquid is filtered into the same capsule and gently evaporated at about 15° C. To the residue 5 c.c. of alcohol and 100 grms. of a saturated aqueous solution of quinine sulphate are added, followed by 10 drops of 1 per cent aqueous hæmatoxylin solution. The capsule is then placed on the boiling water bath to drive off the ether and alcohol. Meanwhile 2 or 3 c.c. of 10 per cent sulphuric acid is added, then gradually a little 5 per cent acid until the liquid assumes a lemon-yellow tint. If this faint acidity requisite be exceeded, a few drops of dilute ammonia are added until only a faint yellow colour is visible. The solution is then set aside in a cool place for twenty-four hours, and the crystals which have formed are collected on a tared filter, washed first with saturated quinine sulphate solution, then with a few c.c. of distilled water used in portions. The mixed sulphates of quinine and cinchonidine are then dried and weighed; 0.75 gm. of these sulphates is then weighed off, dissolved by boiling in 85 c.c. of saturated solution of pure quinine chromate, and treated with 0.20 gm. of pure K_2CrO_4 dissolved in a little water, allowed to cool, and the precipitated quinine chromate collected on a small tared filter, and washed with saturated solution of quinine chromate to bring the volume of the filtrate to 100 c.c. This filtrate may be tested for cinchonidine by the addition of NaOH solution. The crystals are then slowly washed with another 100 c.c. of saturated solution of quinine chromate, drained, dried at 100° C., and weighed as $(C_{20}H_{24}N_2O_2)_2CrO_4$. Since 0.746 gm. of pure quinine sulphate gives under these conditions 0.764 gm. of chromate, the equivalents may be taken as practically 75 and 76. If the first filtrate from the precipitated chromate gives no precipitate with NaOH, the amount of free chromate in the liquid may be determined volumetrically by means of KI and sodium thiosulphate. In this case, a solution of 0.2 gm. of K_2CrO_4 in 154 c.c. of water may be conveniently used as the precipitant; each c.c. of this will be equivalent to 0.005 gm. of anhydrous quinine sulphate. At the same time the amount of thiosulphate used up by the iodine liberated by 100 c.c. of saturated

SEPARATION OF CINCHONA ALKALOIDS.

A weight of not less than 2, and preferably 5, grammes of the mixed alkaloids in a free state is finely powdered,¹ and treated in a closed tube with ten times its weight of ether (free from alcohol). The mixture is well shaken and left at rest for twelve hours, when it is filtered, and the residue washed with a small quantity of ether.

A. The residue is dried and weighed. It may contain *cinchonine*, *cinchonidine*, and *quinidine*. It is dissolved in a slight excess of dilute acetic acid, and the solution rendered neutral to litmus by the cautious addition of soda. The cinchonidine is then precipitated as tartrate, the quinidine as hydriodide, and the cinchonine as the free alkaloid. (See below.)¹

B. The ethereal solution is evaporated to dryness, and the residue weighed. It consists of *quinine*, *amorphous alkaloids* and *quinamine*, with heavy traces of *quinidine* and *cinchonidine*. It is dissolved in 10 parts of proof spirit, acidulated with $\frac{1}{5}$ of sulphuric acid. To this solution an alcoholic solution of iodine is gradually added as long as a precipitate is produced. Excess of iodine must be carefully avoided. In presence of much quinine, a black precipitate of herepaphite is immediately produced, but if the quantity is small, some time is required for its appearance. In such a case only a small quantity of iodine solution must be added, and the liquid well stirred, and left twelve hours. The precipitate is filtered off, and washed with strong alcohol.

C. The precipitate consists of herepaphite. It is dried at 100° C., weighed. The weight, multiplied by .55035, gives the quantity of *quinine* in the mixed alkaloids operated upon. The precipitate may also be treated with sodium thiosulphate or by sulphurous acid and the alkaloid liberated by ammonia, extracted with ether, and titrated or weighed.

D. The solution is treated with sulphurous acid till colourless, and then carefully neutralized with caustic soda. The alcohol is evaporated off, and the liquid treated with excess of soda or ammonia, and agitated with chloroform. The residue left on evaporating the chloroform consists of *amorphous alkaloids*, with considerable traces of *quinidine* and *cinchonidine*. The two latter will remain undissolved on treatment with a limited quantity of ether, and the amorphous alkaloids may be examined by De Vrij's test.

¹ It is not always an easy matter to obtain the mixed alkaloids in a condition of fine powder, especially if their total amount has been determined by evaporating a chloroformic or other solution in a flask. For the treatment of such residues, E. K. Squibb ("Ephemeris," I. 111) recommends that 5 grammes of glass, which have been reduced in a mortar to a mixture of specific gravity not higher than 0.725. The flask is corked and shaken vigorously so as to grind up the alkaloidal residue and mix it thoroughly with the glass. Then pass the liquid through a very small filter (2½ inches) previously wetted with ether, and wash with drops of ether delivered from a pipette till the filtrate measures 10 c.c. Change the receiver and continue the washing till another 10 c.c. have been collected. Then evaporate each portion to dryness, and correct the weight left by the first (quinine, etc.) by that of the residue from the second, which represents the traces of quinidine, cinchonidine, etc., soluble in 10 c.c. of ether.

SEPARATION OF CINCHONINE, CINCHONIDINE, AND QUINIDINE.

The mixed alkaloids extracted from the bark, after dissolving the quinine with ether, or from the filtrate from the crystallized quinine sulphate by treatment with soda and chloroform, are dissolved in dilute sulphuric acid, and the solution exactly neutralized by soda. A saturated solution of Rochelle salt is next added in excess, the liquid cooled to 15° C., and repeatedly stirred during one hour. Crystalline streaks in the track of the glass rod consist of cinchonidine (or quinine) tartrate. The precipitate is collected on a double tared filter, and washed first with a five per cent solution of Rochelle salt and then with a little cold water, the filtrate and washings being collected in a graduated cylinder.

The precipitate is dried at 100° to 105° C. and weighed, the outer filter being used as a counterpoise. The amount found is corrected by adding ·00083 gramme for each 1 c.c. measured by the filtrate and wash-water. The sum multiplied by 0·797 gives the weight of *cinchonidine*. If quinine has not previously been separated, the amount of crystallized sulphate found must be multiplied by ·915, and the product subtracted from the weight of the tartrate before calculating it to cinchonidine. A preferable plan is to dissolve the precipitate off the filter with dilute sulphuric acid, add ammonia, and extract with ether, weighing or titrating the alkaloid.

A. The filtrate is concentrated to its original bulk, cooled, a drop of dilute acetic acid added, and then excess of a saturated solution of potassium iodide (free from any alkaline reaction). The liquid is left for two hours at 15° C., being frequently stirred. Any streaks in the track of the glass rod are produced by quinidine hydriodide. The liquid is filtered on a double counterpoised filter, and the precipitate cautiously washed with cold water.

The precipitate is dried at 100° and weighed. Its weight is corrected by the addition of ·00077 gramme for each 1 c.c. of filtrate and washings (B). The sum, multiplied by ·7168, gives the *quinidine*; or the precipitate may be decomposed by ammonia, the alkaloid extracted by ether, and titrated or weighed.

B. Filtrate is measured and made distinctly alkaline with caustic soda, and the precipitated *cinchonine* is extracted by agitation with chloroform, which is separated, evaporated, and the residue weighed or titrated. The weight found is corrected by deducting ·00052 for each 1 c.c. measured by filtrate A, and ·00086 for each c.c. of filtrate B. Any *amorphous alkaloid* may be dissolved out by spirit of 0·94 specific gravity.

solution of quinine chromate is noted; this number will be β . Operating on the above quantities, the first 100 c.c. of chromate filtrate collected will contain the equivalent of 4 c.c. of the titrated solution of K_2CrO_4 . If this filtrate requires x c.c. of thiosulphate to titrate the iodine it liberates, the amount of quinine sulphate present in the mixed sulphates may be found from the formula $75 - \frac{x - \beta + 4}{2}$ = the number of centigrams present in the 0.75 gm. of sulphates taken.

Quinine $C_{20}H_{24}N_2O_2$, $3H_2O$ is official in the British Pharmacopœia in the form of its hydrochloride $C_{20}H_{24}N_2O_2$, HCl , $2H_2O$; acid hydrochloride $C_{20}H_{24}N_2O_2$, $2HCl$, $3H_2O$; and sulphate $[(C_{20}H_{24}N_2O_2)_2H_2SO_4] \cdot 15H_2O$.

The pure alkaloid contains 14 per cent of water of crystallization which is lost at 120° to 125° , the anhydrous alkaloid melting at about 175° . It should be practically free from cinchonine and cinchonidine, which is assured by 1 gm. dissolving on warming in 6 c.c. of absolute alcohol and 3 c.c. of ether, and remaining perfectly clear when the solution is cooled.

The following typical reactions are characteristic of the base or its salts. To 1 c.c. of a 1 per cent solution in water containing sufficient H_2SO_4 to dissolve the alkaloid, a small quantity of bromine water is added, and then a little dilute ammonia. An emerald-green colour results (thaleoquin reaction). If 0.05 gm. be dissolved in 5 c.c. of alcohol with 5 c.c. of dilute sulphuric acid, and a little acetic acid, and 5 c.c. of a saturated solution of iodine in alcohol be added to the former solution heated to boiling-point, bronze or olive-green crystals of quinine iodo-sulphate will separate on cooling (Herapath's reaction).

Quinine hydrochloride contains 9 per cent of water which it loses at 100° ; 9.2 per cent of hydrochloric acid, which is determined as silver chloride in the usual manner; and 81.7 per cent of anhydrous quinine. This is determined by dissolving 1 gm. in slightly acidulated water, rendering alkaline with K_2CO_3 , extracting with ether and weighing the residue.

The acid hydrochloride should contain 12 per cent of water of crystallization; 16.2 per cent of hydrochloric acid, and 71.86 per cent anhydrous quinine.

Quinine sulphate is the "quinine" of commerce, and is by far the most important form in which the alkaloid is found. By drying at 100° it should lose not more than 15.3 per cent of water of crystallization; and should contain 11.12 per cent of sulphuric acid, which is determined by dissolving 1 gm. in slight excess of dilute hydrochloric acid and precipitating in the usual manner with barium chloride. It contains 73.55 per cent of anhydrous quinine, which is determined as described under the hydrochloride. The Pharmacopœial standards of quinine sulphate are somewhat stringent. It is directed not to afford any appreciable reaction characteristic of cinchonine, quinidine, cupreine or amorphous alkaloid, and should not yield more than 3 per cent of (impure) cinchonidine when tested as follows:—

Test for Cinchonidine and Cinchonine.—Four grms. are dissolved

in 120 c.c. of boiling water. The solution is cooled to 50° C., with constant stirring. The sulphate of quinine which crystallizes is separated by filtration. The filtrate is reduced to 10 c.c. by evaporation. When cold it is shaken with 10 c.c. of ether and 5 c.c. of ammonia (specific gravity = 0.959). The whole is set aside in a stoppered vessel for twenty-four hours. The crystals separating are collected on a tared filter, washed with ether, dried at 100° and weighed. They consist of cinchonidine, cinchonine and a little quinine, and should not weigh more than 0.12 grm.

Test for Quinidine.—One grm. is dissolved in 30 c.c. of boiling water, the solution is cooled and weighed. Solution of potassium iodide and a little alcohol are added. Any quinidine hydriodide is collected, washed with a little water, and weighed. Not more than the slightest trace should be obtained.

Test for Cupreine.—The recrystallized sulphate of quinine, obtained in testing for cinchonidine, is shaken with 25 c.c. of ether and 6 c.c. of ammonia (specific gravity 0.959). To the separated ethereal liquid, add the ethereal liquid and washings obtained in testing for cinchonidine, and add 6 c.c. of a 10 per cent solution of NaOH, adding water if any solid matter should separate. Remove the ethereal liquid, treat the aqueous liquid with more ether and remove the ethereal washings. Heat the aqueous liquid to boiling and neutralize with dilute sulphuric acid when cold, collect any sulphate of cupreine that may have separated, on a tared filter. Only the slightest traces should be found.

Test for Cinchonine and Amorphous Alkaloid.—Dissolve 1 grm. of quinine sulphate in 30 c.c. of boiling water, and add 1 grm. of sodium potassium tartrate. Allow to cool, with frequent stirring; filter. The filtrate when evaporated to a small bulk should give little or no precipitate with solution of ammonia.

The Detection and Determination of Quinine.—One of the most definite indications of the presence of quinine is its fluorescence in a dilute sulphuric acid solution. This property is impaired by the presence of chlorides and other salts, but is under most conditions still observable in the presence of excess of sulphuric acid. In the presence of other organic matter, quinine is extracted by rendering the mass alkaline with potassium carbonate and thoroughly extracting with ether. The ethereal liquid is extracted with dilute sulphuric acid, and in the presence of quinine, this will show a marked blue-violet fluorescence and have a typically bitter taste. The presence of quinine may be confirmed by the thalleoquin reaction (see quinine sulphate, p. 532), but it must be remembered that other cinchona alkaloids yield this reaction. It may also be confirmed by Herapath's reaction (p. 532).

If the alkaloid be extracted by ether from an alkaline mass, the ether washed with water, evaporated, and the residue weighed, an approximate determination results, or the residue may be dissolved in excess of decinormal sulphuric acid and the excess of acid remaining may be titrated with decinormal soda, each c.c. used being equivalent to 0.0324 grm. of anhydrous quinine.

Vigneron's process (p. 529) is applicable to the determination of quinine, when the alkaloids present in the substance to be examined have been extracted by ether from the mass previously rendered alkaline.

Iron and Quinine Citrate is an official preparation. It occurs as brownish-green scales soluble in water. It should not contain more than 11 per cent of water and should yield an ash which is not alkaline to litmus; the official standard is that it should contain 15 per cent of quinine, as determined by rendering an aqueous solution alkaline with ammonia and extracting with ether, and drying the residue at 120°. The ash value is from 18 to 20 per cent which should consist almost entirely of Fe_2O_3 .

Liquid Extract of Cinchona.—This official preparation is a liquid extract made by percolating the powdered bark with a mixture of water and glycerin with a little hydrochloric acid, and adding alcohol to the concentrated percolate. The official standard is that it should contain 5 grms. of alkaloids per 100 c.c., when assayed in the following manner:—

Five c.c. and 25 c.c. of water are well shaken in a separator with 30 c.c. of a mixture of 3 volumes of benzol and 1 volume of amyl alcohol, and 15 c.c. of a 10 per cent solution of potash. Run off the lower aqueous layer, and well wash this with 30 c.c. of the same solvent, and mix the two portions of solvent. Wash with water and then shake well with 30 c.c. of 2 per cent HCl, separate the acid liquid and repeat the extraction. Mix the acid liquids. Render alkaline with ammonia and extract three times with 10 c.c. of chloroform. Evaporate the chloroform and dry the residue at 110° and weigh. A genuine liquid extract of cinchona should have the following characters:—

Specific gravity	1.115 to 1.180
Solid residue	88 „ 48 per cent
Alcohol by volume	11 „ 18 „

There are four tinctures of cinchona or quinine official in the British Pharmacopœia, which should have the following characters:—

	Specific Gravity.	Solid Residue.	Alcohol by Volume.	Quinine Gr. per 100 c.c.
Tincture of cinchona .	0.914 to 0.924	6.2 to 6.9	Per cent 63	0.95 to 1.05 ¹
Compound tincture of cinchona	0.914 „ 0.924	4.6 „ 5.2	65	0.45 „ 0.55 ¹
Tincture of quinine .	0.885 „ 0.893	3.5 „ 3.9	74	1.634 ²
Ammoniated tincture of quinine	0.925 „ 0.930	1.8	54	1.471 ³

¹ Official standards: to be determined as described under liquid extract of cinchona.

² In the form of hydrochloride, which should be present to the extent of 2 grms. per 100 c.c.

³ As quinine sulphate (2 grms. per 100 c.c.). NH_3 should be present to the extent of about 1 per cent to 1.03 per cent.

Quinine Wine.—This galenical is official and is a solution of 20 grains of quinine hydrochloride in 20 fluid ounces of orange wine. The official requirements are to be deduced from the directions given for its preparation. It should have the following characters:—

Alcohol by volume	10 to 12 per cent
Quinine as alkaloid	·187 grm. per 100 c.c.

It should contain no salicylic acid, which is detected by acidifying with sulphuric acid, distilling off the alcohol, and then extracting the aqueous distillate with ether and testing the ether residue with ferric chloride, when no violet colour should result.

COCA.

The dried leaves of *Erythroxylon Coca* (and its varieties) are official in the Pharmacopœia, and also a liquid extract, but no official standards are given.

The principal constituent of the leaves is the alkaloid cocaine $C_{17}H_{21}NO_4$, which is described below; this alkaloid is methyl-benzoyl-ecgonine, and is associated in the leaves with the bases truxilline (also known as cocamine) $C_{38}H_{46}N_2O_8$ which is iso-atropyl-cocaine; methyl-cinnamyl-ecgonine $C_{19}H_{23}NO_4$, and tropococaine $C_{17}H_{19}NO_2$. Other alkaloids are present in traces, nearly all of them being derivatives of ecgonine $C_9H_{15}NO_3$. The leaves contain from 0·2 per cent to 1·1 per cent of alkaloids, the Peruvian variety, known as Truxillo leaves, usually containing more alkaloid than the Bolivian leaves, but only about 50 per cent of the total is cocaine, whereas the alkaloids of the Bolivian leaves contain about 80 per cent of cocaine.

Coca leaves contain from 6 per cent to 8 per cent of mineral matter. The only determination necessary with this drug, which is practically always met with in the whole condition, is the alkaloidal value.

A microscopic examination, however, may be made of the powder when necessary.

Prismatic crystals of calcium oxalate are present, and characteristic papillose cells on the lower epidermis. Sclerenchymatous fibres, and pitted and spiral vessels, are present in numbers. The sketch on page 536 represents powdered coca leaves.

The cocaine may be determined in coca leaves by one of the following processes:—

Pfeiffer digests 100 grms. of the powdered leaves with 400 c.c. of water, 50 c.c. of a 10 per cent solution of soda, and 250 c.c. of light petroleum. The whole is kept at a temperature of about 40° C. for three to four hours with occasional shaking, and then strained and the residues pressed. There is no fear of the petroleum being retained by the leaves to any extent, as it is sharply separated as an oily layer on the watery solution. The aqueous layer is run off and the oily layer titrated with $\frac{N}{10}$ hydrochloric acid, of which 1 c.c. is equivalent to 0·0303 grm.

Methyl orange, or tincture of Cochineal, may be used as indicator.

Lyons recommends that the finely powdered leaves should be macerated for twenty-four hours with eight times their weight of a mixture of 95 volumes of ether and 5 of ammonia. From an aliquot part of this liquid the alkaloid is extracted by agitation with acidulated water, the ether separated and the alkaloid liberated from the aqueous liquid by means of ammonia and again extracted with ether, which is then evaporated and the cocaine weighed. The other alkaloids are soluble in water, but insoluble in ether, so do not interfere with the determination of the cocaine. Gunn prefers to moisten 5 grms. of the

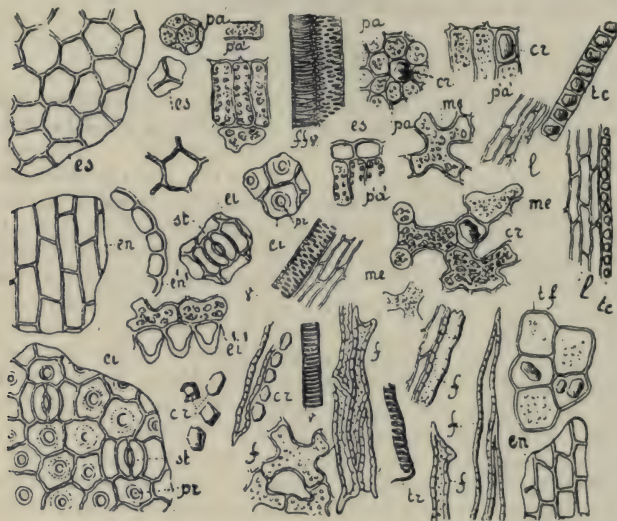


FIG. 48.—Powdered coca leaves $\times 240$. *cr*, prismatic crystals of calcium oxalate; *ei*, lower epidermis, with surface view of papillose cells (*pr*); *e's*, lower epidermis in section; *f*, sclerenchymatous fibres; *ffv*, fragments of vessels from midrib; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a*, palisade cells; *st*, stomata, with two subsidiary cells parallel to the ostiole; *tc*, crystal cells; *tf*, cortical tissue of midrib; *tr*, vessels, etc. (Greenish & Collin.)

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powdered leaves with dilute ammonia and after allowing them to stand for thirty minutes to proceed as follows:—

They are then placed in a narrow tubular percolator (10 inches long and of $\frac{1}{2}$ -inch bore) and percolated with ammoniated ether until 100 c.c. are collected. This is shaken out with three washings by a 2 per cent solution of hydrochloric acid, collecting about 50 c.c. of the washings. This acid solution is now washed once with ether, then made alkaline with ammonia, and the alkaloid shaken out with three washings of ether. The collected portions of ether are transferred to a weighed porcelain dish, the ether blown off, and the residue dried at 75°C .

When the bases of coca leaves have been extracted with alcohol (as is the case with much crude cocaine) the cocaine may be determined

by dissolving the mixed alkaloids in the minimum quantity of dilute hydrochloric acid and then using the process of Garsed and Collie ("Proc. Chem. Soc." xvii. 89). Advantage is taken of the fact that cocaine forms a very stable insoluble di-iodohydriodide $C_{17}H_{21}NO_4HI_2$, so that by adding an excess of decinormal iodine to a solution containing a salt of cocaine, and then titrating the excess of iodine in the usual manner, the amount of cocaine may be determined, or the di-iodo-compound may be collected and weighed. Ecgonine does not interfere with the results since it forms soluble iodo-compounds. Benzoyl-ecgonine, however, interferes, and should be removed by treating the liberated bases with petroleum ether, or ether in which only cocaine is soluble.

Liquid Extract of Coca is an extract of the drug by 60 per cent alcohol, of such strength that 1 fluid ounce of the extract contains the soluble matter of 1 ounce of the drug.

A properly prepared extract should have the following characters :—

Specific gravity	=	0.995 to 1.031
Solid residue	=	18 „ 20 grms. per 100 c.c.
Alcohol by volume	=	49 „ 52 per cent
Cocaine	=	0.2 „ 0.6 „

This preparation should be of certain alkaloidal strength, and the fact that the leaves contain so variable an amount of cocaine renders it probable that the next edition of the British Pharmacopœia will, as in many other cases, require the extract to contain a definite proportion of cocaine.

For the determination of the cocaine the following process may be employed, which is due to Garsed :—

One hundred c.c. are evaporated to 50 c.c. in a shallow dish on a water bath, at a temperature never exceeding 80° C. with constant stirring, to remove alcohol. When cold, the extract is made alkaline by the addition of 5 c.c. of 10 per cent ammonia, and transferred to a separating funnel. The dish is washed out first with 45 c.c. of distilled water, then with 100 c.c. of ether. The water and ether washings are added to the rest in the separator, the whole well shaken and allowed to stand until the ether separates, when the alkaline liquid is drawn off. The extraction with ether is three times repeated. Four ether solutions are thus obtained. The first three are mixed together, washed with a few c.c. of water containing a little ammonia, and shaken out first with 5 c.c. of 5 per cent sulphuric acid, then twice with 5 c.c. of 1 per cent acid. This is sufficient to completely exhaust the ether solution, the test being the addition of a few drops of Mayer's reagent to the last few drops of the third quantity of acid, when, as a rule, no precipitate or opalescence is produced. The three acid solutions are mixed together, made alkaline by the addition of 10 per cent ammonia, and three times shaken out with 10 c.c. of petroleum ether, the bulked petroleum-ether extract evaporated to dryness on a water bath in a tared dish, then placed in a desiccator for some hours, and finally weighed.

Cocaine.—Both cocaine and its hydrochloride are official in the Pharmacopœia. The following are the official requirements for the alkaloid and its salt :—

Cocaine.—The alkaloid should melt at 96° to 98° C. It is almost insoluble in water, insoluble in glycerine, soluble in 10 parts of 90 per cent alcohol, in 4 parts of ether, in 0.5 part of chloroform, in 12 parts of olive oil and in 14 parts of turpentine. Its solution in dilute nitric acid should give no reactions for sulphates or chlorides, and its solution in dilute hydrochloric acid, when evaporated to dryness, should give the reactions described under the hydrochloride.

Cocaine Hydrochloride.—The British Pharmacopœia requires that this should melt at 180° to 186° C. The U.S.P. gives 193° as the melting-point of the pure substance. It is soluble in half its weight of cold water, forming a clear colourless solution of neutral reaction; and in four times its weight of 90 per cent alcohol, or glycerin. It is insoluble in olive oil and nearly so in ether. It gives a yellow precipitate with auric chloride solution, and a white precipitate with ammonium carbonate or borax solutions. It dissolves without colour in cold H_2SO_4 or HNO_3 , but chars with hot sulphuric acid, yielding a sublimate of benzoic acid. An aqueous solution yields a white precipitate with potassium hydroxide solution, which is soluble in alcohol and ether; and a yellow precipitate with solutions of picric acid; with solutions of HgCl_2 it gives a white precipitate in solutions slightly acidified with HCl , which is soluble in hot water. If a fragment be moistened with HNO_3 , evaporated to dryness and a drop of alcoholic potash solution added, a characteristic odour recalling that of peppermint is evolved. A solution of not less than 1 per cent strength gives, with excess of potassium permanganate, a copious red precipitate which does not change colour within an hour (absence of cinnamyl-cocaine (*so called*), and cocamine, etc.) If 0.1 grm. be dissolved in 100 c.c. of water and 0.25 c.c. of a solution of ammonia (10 per cent) be added, a clear solution should result, from which a crystalline deposit should gradually separate on stirring. It should contain no sulphates. Dried at 100° it should not lose more than 1 per cent of moisture, and it should contain no mineral matter.

Pure cocaine melts at 98° and is lævorotatory, the specific rotation in chloroform solution being about -16° , whilst that of the hydrochloride in alcoholic solution is about -70° .

Synthetic cocaine, that is, cocaine made by hydrolysing allied alkaloids of little or no therapeutic value, which yield *l*-ecgonine, is made by some manufacturers. This is benzoylated and methylated, and the resulting product is identical with natural cocaine. A synthetic, optically inactive, cocaine is made in a similar manner from inactive synthetic ecgonine. Cocaine has the constitution of a methyl-lævo-benzoyltropine carboxylate. It gives a rose-coloured precipitate with a solution of iodine in iodide of potassium, or if the solution be strong, the precipitate is brown. If a drop of ferric chloride be added to a solution of cocaine and the liquid boiled, an intense red colour is developed. The usual alkaloidal precipitants

yield precipitates with cocaine, phosphomolybdic acid being one of the most delicate reagents with this alkaloid.

For the distinctions between Cocaine and similar local anaesthetics see Hawkin ("Analyst," xxxvi. 2).

The Examination of Cocaine.—Cocaine can readily be obtained in a state of great purity. In addition to the official test given above, cocaine should comply with the following requirements, the alkaloid being usually examined as the hydrochloride. The optical activity of the hydrochloride should be taken in dilute alcoholic solution, and at 20° its specific rotation (see under sugars) should, in 2 per cent aqueous solution, be -71° , or, in 40 per cent alcohol -69° (Antrich, "Berichte," xx. 310).

The specific rotation -52° which is frequently given in text-books, is erroneous, and arises from a misinterpretation of Antrich's equation.

If 0.1 grm. of the hydrochloride be dissolved in 5 c.c. of water, and 3 drops of dilute H_2SO_4 be added, and then 1 drop of a 1 per cent solution of potassium permanganate added, the liquid, kept in a closed vessel, should only slightly decrease in colour in thirty minutes.

MacLagan proposed the following test: One grain of the salt is dissolved in two ounces of water, two drops of strong ammonia solution (880 specific gravity) are added and the walls of the vessel rubbed from time to time with a glass rod: in fifteen minutes a good crop of glistening crystals separate. If the cocaine be not very pure either no crystals appear, or at most only a slight crop. If more than four per cent of amorphous alkaloid be present, the liquid becomes milky.

B. H. Paul ("Pharm. Jour." 3, xviii. 783) has improved this test. He adds ammonia gradually with constant stirring to a 2 per cent solution of the salt, as long as a crystalline precipitate forms and the liquid clears quickly. Directly clots begin to be precipitated the crystalline precipitate is filtered off and the amorphous precipitate produced by adding more ammonia is collected separately. Calculated in the dry salt, the crystalline precipitate should weigh not less than 82 per cent to 84 per cent.

Cocaine and its hydrochloride should not contain more than 1 per cent of moisture.

COLCHICUM.

The corms of *Colchicum autumnale*, as well as the seeds, are official. An extract of the fresh corms, a wine prepared from the corms, and a tincture of the seeds are all official, but no standards are given for any of them.

The active constituent of both the corms and the seeds is the toxic alkaloid colchicine $\text{C}_{22}\text{H}_{25}\text{NO}_6$, which is present in the corms to the extent of 0.4 per cent to 0.65 per cent and in the seeds to the extent of 0.6 per cent to 0.8 per cent.

Colchicum seeds contain from 4 per cent to 5 per cent of mineral matter, and the corms from 2 per cent to 3 per cent.

Assay of Colchicum Seeds.—Farr and Wright's process ("Pharm. Jour." Vol LXXXV. 1910, p. 579). Pack 5 grms. of the seeds in No.

30 powder in a glass tube about 2 cm. diameter and exhaust by slow percolation with 50 per cent alcohol. Transfer the percolate to a porcelain dish add 25 c.c. water and evaporate to about 20 c.c. over a water bath. Transfer to a separator, rinsing the dish first with a little water and then with 25 c.c. petroleum ether, add the rinsings to the separator and shake vigorously. When the liquids have separated reject the upper layer, return the residual liquid to the separator, and twice repeat the washing with 20 c.c. of petroleum ether. Saturate the aqueous liquid with sodium chloride and shake vigorously with 20 c.c. of chloroform. Twice repeat the extraction with 10 c.c. of chloroform and mix the chloroform solutions. Recover the chloroform and treat the residue first with a mixture of 19 c.c. water and 1 c.c. solution of ammonia, used in four portions and then with a mixture of 16 c.c. water and 4 c.c. diluted sulphuric acid. Strain the solutions through cotton wool into a flask, shake, add 20 c.c. of decinormal solution of iodine, set aside for 5 minutes, and collect the precipitate on a small filter, washing the flask and precipitate with 20 c.c. of distilled water containing 1 c.c. decinormal iodine and 1 c.c. of dilute sulphuric acid. Drain the filter, then place it in a small mortar with 2 c.c. of sodium carbonate test solution and 20 c.c. of decinormal solution of sodium thiosulphate until the filter has been reduced to a pulp. Filter the mixture through cotton wool into a separator, rinse the mortar with several small portions of distilled water until a few drops of filtrate acidulated with dilute sulphuric acid cease to give a precipitate with a few drops of iodine solution.

Shake the liquid in the separator vigorously for 1 minute with 20 c.c. of chloroform, and draw off the chloroform into a tared platinum dish, repeat the process twice with 10 c.c. chloroform and evaporate the chloroform solutions to dryness at a low temperature. Dissolve the alkaloids in a little 90 per cent alcohol, evaporate over a water bath and dry at 100° to constant weight.

The alkaloid obtained by this process is a very pale straw colour perfectly soluble in water. Dissolved in chloroform and poured into excess of petroleum ether, the alkaloid is precipitated quantitatively in a nearly colourless condition.

For the determination of Colchicine, Lyons ("American Druggist and Ph. Record," Feb. 1909) uses the following gravimetric method which gives accurate results:—

Place in a small beaker 25 grms. of colchicum corm in moderately fine powder. Add 15 c.c. of solution of lead subacetate, and 80 c.c. of warm distilled water, and macerate with occasional stirring for six hours at a temperature of about 50° C. Transfer to a small percolator (a funnel answers the purpose well), having the tube so packed that percolation will go on at the rate of about 2 c.c. per minute. When the fluid has disappeared from the surface of the drug add warm water, about 20 c.c. at a time, and so continue the percolation until 250 c.c. of fluid has been collected. This should practically exhaust the drug. To the percolate add 5 grms. of powdered sodium phosphate, or enough to precipitate the whole of the lead present in the percolate. Filter, returning the first portion of filtrate if it is not quite clear.

Use for duplicate assays two portions, 100 c.c. each of the filtrate, representing 10 grms. of drug. Shake out each portion with three successive portions of chloroform, 25, 20, and 15 c.c. or enough to extract the whole of the colchicine. Evaporate off the chloroform and treat the residual alkaloid repeatedly with 90 per cent alcohol to remove persistently adhering traces of chloroform, dry at a temperature below 100° C. to constant weight. The weight of the alkaloid in grms. multiplied by ten gives the percentage of colchicine in the drug. This may be confirmed by titration with Mayer's reagent. The alkaloid will be found to be remarkably free from impurities.

The more recent method is that of Farr and Wright (p. 539).

Tincture of Colchicum is a 45 per cent alcohol extract of the seeds, of which 4 ounces are used for 1 pint and should have the following characters :—

Specific gravity	=	0.950 to	0.960	
Solid residue	=	1.90	„	2.4 grms. per 100 c.c.
Alcohol by volume	=	41	„	43 per cent
Alkaloids	=	0.05	„	0.09 „

Vinum Colchici.—This preparation is made by macerating 4 ounces of colchicum corms in powder in 1 pint of sherry. It should contain from 14 per cent to 15 per cent of alcohol by volume, and should be free from salicylic acid (see quinine wine, p. 535).

Colchicine.—The alkaloid colchicine $C_{22}H_{25}NO_6$, and its salicylate $C_{22}H_{25}NO_6 \cdot C_7H_6O_3$ are met with in medicine and are frequent constituents of gout remedies.

Colchicine is an amorphous powder, soluble in water, alcohol, and chloroform. It melts at 145°. A minute quantity dissolved in sulphuric acid, gives with nitric acid a rich greenish-blue colour, which changes to pale blue and then to red and yellow. The yellow solution is turned red by caustic soda solution. Nitric acid gives a dirty violet colour, passing to greenish and then to yellow. An alcoholic solution gives a garnet red colour with ferric chloride. In organic mixtures suspected of containing colchicine it is easily precipitated by phosphomolybdic acid, and the resulting precipitate may be treated with ammonia and the free alkaloid extracted by chloroform, when it will give the foregoing reactions.

COLOCYNTH.

The dried pulp of the fruit of *Citrullus colocynthis*, free from the seeds, is the official drug of the British Pharmacopœia.

The official standards are as follows : It should not yield a reaction for starch, and should only yield traces of fixed oil to ether. It should yield not less than 9 per cent of ash.

Numerous bodies have been described as active principles of this drug, but the recent researches of Power and Moore ("Chemist and Druggist," 1910, I. 150), have corrected many of the erroneous earlier statements. These chemists have isolated a dihydric alcohol $C_{22}H_{36}O_2$ $(OH)_2$ which they have termed citrullol (apparently a homologue of ipuranol). A very small quantity of an alkaloid was obtained, which

neither crystallizes nor yields crystalline salts, but which has a powerful physiological action. Traces of a glucoside are also present and about 1 per cent of α -elaterin. The results of this investigation have established the fact that the so-called "colocynthin," "colocynthinin," and other products heretofore obtained from colocynth to which specific names have been attached, are not pure substances, but very indefinite mixtures, and that the amount of glucosidic substance present is extremely small. On the other hand, it has been shown that the activity of colocynth is due to at least two principles, one of which is alkaloidal, although a very weak base, whilst the other source of activity is represented by some non-basic principle or

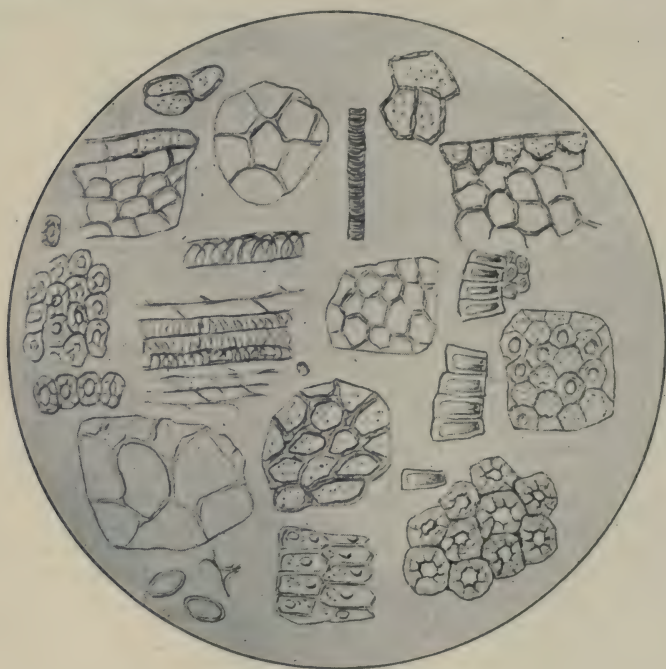


FIG. 49.—Powdered colocynth.

principles contained in the ether and chloroform extracts of the resin, but which did not permit of being more definitely characterized. The colocynth contains, furthermore, a quantity of α -elaterin, but no evidence could be obtained of the presence of β -elaterin, which is the physiologically active constituent of the fruit of *Ecballium elaterium*.

According to the British Pharmaceutical Codex, the ash should vary between 7 per cent and 13 per cent, but it is generally maintained that 10 per cent to 13 per cent is the better standard. Not more than 1.5 per cent of fixed oil should be extracted by petroleum ether.

The indefinite nature of the active principles of this drug render any process of assay of doubtful value. The following process, however, due to Brautigam ("Journ. Pharm. Chim." 6, 16, 130) has in the author's hands given concordant results on a number of samples examined. Three grms. of the pulp are thoroughly exhausted with water, and the water evaporated. The residue is then extracted with two successive quantities, each of 30 c.c. of 90 per cent alcohol, for one hour at 20° to 25° C., with frequent agitation. The residue is washed with 20 c.c. of alcohol. The bulked alcoholic solution is filtered and evaporated to dryness. The residue is triturated with water, made up to about 120 c.c. and left in contact for twenty hours at 25° C., with frequent and thorough agitation. The mixture is then filtered, the filter washed with 20 c.c. of water, and then 0.25 gm. of lead acetate is dissolved therein, and 3 grms. of 5 per cent solution of basic lead acetate added. When precipitation is complete, the precipitate is filtered off and washed with two portions, each of 30 c.c. of water. To the filtrate, aluminium sulphate 2 grms., and animal charcoal 4 grms., are added, and the mixture is evaporated to dryness. The residue is taken up with two successive 30 c.c. of ether, and the ethereal extract evaporated. The residue is macerated twice in succession, each time for one hour, with alcohol 40 c.c., and finally washed with another 30 c.c. The bulked alcoholic solutions are filtered and evaporated to dryness. The residue is taken up with a little absolute alcohol, and filtered through a small filter, previously moistened with alcohol, the filtration being repeated until the liquid is quite bright. The filter is washed with a little absolute alcohol; the bulked liquids are evaporated in a small tared capsule. The residue is dried to constant weight, and weighed. It should not be less than 0.04 gm., and should be completely soluble in 2 c.c. of absolute alcohol. On adding 2 drops of this solution to 4 c.c. of ether, a flocculent white precipitate should be obtained; and the same quantity should give with 4 c.c. of water a cloudy solution which precipitates on standing. One or two drops of the "colocynthin" solution, evaporated to dryness at a gentle heat, should give a fine red colour when treated with H_2SO_4 . A similar residue should give a cherry-red colour with Fröhde's reagent; and with sulphuric acid containing 0.5 per cent of ammonium vanadate, a red colour gradually becoming blue at the edge of the liquid, results.

CONIUM.

The fresh leaves and the dried fruits of *Conium maculatum* are official drugs; a juice prepared from the former, and a tincture from the latter, being also official. No standards are given for either the drugs or their preparations.

The active constituent of the leaves is the alkaloid coniine $C_8H_{17}N$ with a certain amount of subsidiary compounds. The amount of alkaloid present, however, rarely exceeds 0.25 per cent, whilst the dried fruits in their best condition contain as much as 3 to 3.5 per cent of coniine. As found in commerce, however, the fruits

rarely contain more than 1 per cent of alkaloid, owing to the fact that they are collected after ripening has commenced.

This is shown in the following table, due to Farr and Wright:—

HYDROCHLORATES OF MIXED ALKALOIDS PER CENT.

	1892.		1893.	
	Fresh.	Dried.	Fresh.	Dried.
Immature, $\frac{1}{4}$ to $\frac{1}{2}$ grown	—	—	—	—
" $\frac{1}{2}$ to $\frac{3}{4}$ "	·975	—	—	—
" $\frac{3}{4}$ to "	—	—	1·049	3·32
Nearly mature, $\frac{2}{3}$ to full grown . .	·985	—	—	—
Mature, $\frac{2}{3}$ to full grown	—	—	1·088	3·36
Mature, a few outer ones beginning to turn slightly yellow	—	—	1·049	—
Mature, yellowish-green to yellow .	·475	—	—	—
Mature, yellow	·434	1·44	—	—
Ripe, grey	—	1·32	—	—

The amount of moisture in the fresh fruit varies from about 60 per cent in the older stages to about 68 per cent in the younger, but is not a constant proportion.

The amount of ash yielded by conium leaves varies from 12 per cent to 15 per cent; whilst that of the seeds lies between the limits 5 per cent and 7 per cent.

A good commercial sample of conium seeds will contain from 0·5 per cent to 1·1 per cent of alkaloid, whereas the leaves rarely contain more than 0·2 per cent.

The best method for the determination of conium alkaloids is that of Cripps.

Cripps ("Pharm. Journ." **3**, xviii. 13, 511) exhausts 5 grms. of the finely powdered fruit (20 grms. of the leaves should be used) mixed with sand, by a mixture of absolute alcohol (25 c.c.), chloroform (15 c.c.), and chloroform saturated with dry HCl gas (10 c.c.). After complete exhaustion the liquid is shaken with 25 c.c. of water twice, the mixed aqueous liquids, containing the alkaloids as hydrochlorides, being then once washed with chloroform, rendered alkaline with soda, and extracted three times with chloroform. The chloroform is washed with a little slightly alkaline water and then run into an ethereal solution of dry HCl gas. The solvent is evaporated in a current of warm air, and the residue dried at a temperature not exceeding 90° C. The hydrochlorides of this alkaloid should be crystalline and practically white. 163·5 parts of hydrochloride contain 127 of coniine (the small amounts of other alkaloids do not materially interfere with this ratio).

This process is improved by titrating the free alkaloid which is in the chloroform after its liberation by means of alkali (and after the chloroform has been washed) with decinormal HCl and methyl orange, until after shaking well the pink colour does not disappear. Each c.c. of

$\frac{N}{10}$ HCl is equivalent to 0·0127 gm. of coniine.

Tincture of Conium is a 70 per cent alcohol extract of the fruits, four ounces of the drug producing one pint of tincture. A properly prepared tincture should have the following characters:—

Specific gravity	0.895 to 0.902
Solid residue	1.3 „ 1.45 grms. per 100 c.c.
Alcohol, by volume	66 „ 68 per cent
Alkaloids as coniine	0.05 „ 0.1 „

Farr and Wright (*“ Pharm. Jour.”* 3, xxi. 857) assay the tincture by evaporating 50 c.c. with 1 c.c. of normal sulphuric acid, down to a low bulk, and shaking the liquid twice with chloroform. It is then rendered alkaline, and the free alkaloids extracted with chloroform three times. The chloroform is freed from traces of alkali by washing with water, separated and run into a solution of dry HCl in chloroform. The solvent is then evaporated and the hydrochloride weighed as in Cripps' process described above.

DIGITALIS.

The leaves of *Digitalis purpurea* are official in the British Pharmacopœia, as well as a tincture, but no standards are given for either. The principal constituents of this drug are glucosides. Much controversy has taken place in reference to the chemistry of these bodies, but the following appears to be now settled.

Digitalin $C_{35}H_{56}O_{14}$, is the principal constituent of the commercial “digitalin” which is generally very impure. It forms fine crystals, but is generally obtained as an amorphous powder. On hydrolysis it yields digitaligenin $C_{22}H_{30}O_3$, glucose, and digitalose, a sugar of the formula $C_7H_{14}O_5$.

It yields a somewhat characteristic reaction when dissolved in a minute quantity of concentrated sulphuric acid and a drop of a solution of potassium hypobromite added: a fine rose or violet-red results. Sulphuric acid containing a trace of ferric sulphate gives at first a yellow colour, changing to red and then to violet-red, which is fairly permanent. It melts at about 217° .

Digitoxin $C_{34}H_{54}O_{11}$, forms colourless crystals, generally very small. It crystallizes from methyl alcohol and chloroform in an anhydrous condition, but from diluted ethylalcohol, with 5 molecules of water of crystallization. The latter form melts at 145° , the anhydrous variety commencing to melt somewhat indefinitely at 240° . On hydrolysis it yields digitoxigenin $C_{22}H_{32}O_4$ and digitoxose $C_6H_{12}O_4$. If a few milligrams are dissolved in acetic acid and a drop of dilute solution of ferric chloride be added, and then concentrated H_2SO_4 be poured down the side of the tube, so as to form a layer under the acetic acid a brownish-green band appears, altering quickly so that the top layer of the sulphuric acid is coloured brownish-red and above this is a broad bluish-green band, which soon becomes indigo blue. After a long time green again appears, and finally fades to a brownish colour.

Digitoxin is the principal glucoside present in the leaves, and is the most reliable preparation to use.

Commercial "digitalin" is frequently a mixture of true digitalin and digitoxin. According to Merck the following are the characters of the principal commercial "digitalins":—

(1) German digitalin; consists principally of digitalein with some digitoxin and digitalin. It is freely soluble in alcohol, but insoluble in ether and chloroform.

(2) Nativelle's crystallized digitalin. This consists almost entirely of digitoxin. It forms fine white needles, insoluble in water, ether, or petroleum ether. It is the type of "French digitalin".

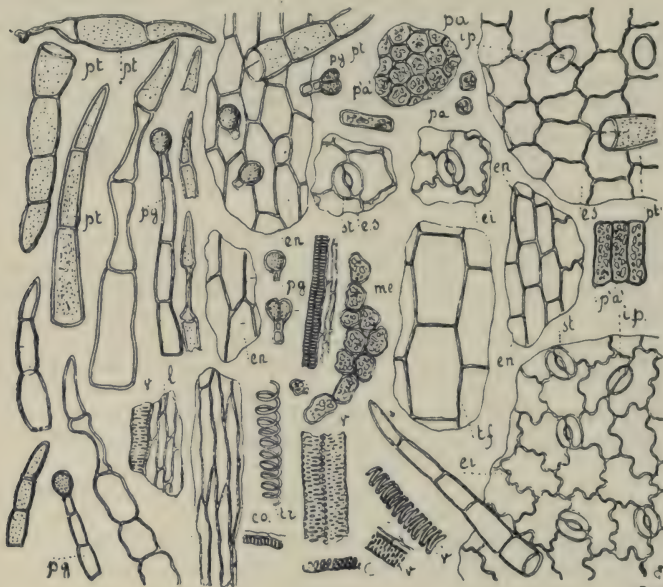


FIG. 50.—Powdered digitalis leaves $\times 240$. *co*, collenchymatous cells of the midrib; *ei*, lower epidermis, cells with sinuous walls; *en*, neural epidermis; *es*, upper epidermis; *ip*, scar of fallen hair; *l*, bast; *me*, spongy parenchymatous; *pa*, *p'a*, palisade cells; *pg*, glandular hairs; *pt*, simple hairs; *st*, stomata; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, etc. (Greenish & Collin.)

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(3) Hommolle's amorphous digitalin. A white or yellowish-white powder, slightly soluble in water and ether, freely soluble in 90 per cent alcohol and in chloroform. It consists principally of digitalin with some digitoxin and corresponds with "French amorphous digitalin".

(4) Pure digitalin Merck. A yellowish-white powder corresponding with No. 1.

A microscopic examination of the leaves in powder shows a lower epidermal tissue with sinuous walls, many single hairs, spiral and pitted vessels and numerous glandular hairs. The above illustration represents the powdered leaves.

Digitalis should not contain more than 10 per cent or at most 11 per cent of ash. The usual amount is 8 per cent to 10 per cent.

The leaves may be assayed as described under the tincture, being first extracted with alcohol. There are, however, advocates of a physiological standardization, on account of the difficulty of deciding the relative proportions and activities of so many nearly related bodies as are present in this drug.

Digitoxin is, as has been mentioned, the principal of these bodies, and Keller ("Ber. Deutsch. Pharm. Ges." 1897, 7, 125) estimates this in the following manner (slightly modified by Barger and Shaw):—

Twenty grms. of leaves, or 146 grms. of tincture, are employed. The British Pharmacopœia directs that 125 grms. of leaves should be percolated with sufficient 60 per cent alcohol to produce 1000 c.c. of tincture. The density of this alcohol is 0.913, hence 1 gm. of leaves

is percolated with $\frac{0.913 \times 1000}{125} = 7.3$ grms. of alcohol. In order to

have quantities equivalent to Keller's 20 grms. of leaves, 146 grms. of tincture may be used for an estimation. The 146 grms. of tincture are evaporated on a water bath to 25 c.c. or less to remove the alcohol, and made up with water to 222 grms. Here the first difficulty presents itself, for by the evaporation of the alcohol a resin separates out, which is mostly insoluble in water, and any digitoxin which may be contained in it will escape estimation. The water is best added in small quantities, and the dish containing the resin warmed on the water bath, while its contents are stirred vigorously, so as to have it suspended in as fine a condition as possible.

To the 222 grms. of turbid solution 25 grms. of a saturated basic lead acetate solution are added, and the precipitate filtered off.

In some cases rather more lead solution is necessary.

A voluminous precipitate results, which is filtered off.

As the total bulk now weighs 247 grms. of which, as experimentally shown, 7 grms. are precipitated, there remains 240 grms. of solution. Of this 132 grms. are easily obtained in the clear filtered condition and represent 11 grms. of leaves. Five grms. of sodium sulphate dissolved in 6 grms. of dilute sulphuric acid are now added. The lead sulphate is filtered off and 130 grms. of the filtrate (= 10 grms. of leaves) are rendered alkaline with 2 c.c. of 10 per cent ammonia; the solution remains perfectly clear, and is shaken out four times with 30 c.c. of chloroform. The chloroform extract is filtered, evaporated to a small bulk, and then washed into a small wide weighing bottle with ground stopper, in which it is evaporated to dryness, first on the water bath, then in the steam oven, till of constant weight. The residue is "crude digitoxin". Keller purifies this by dissolving it again in chloroform, adding ether and petroleum ether, and collecting the precipitate on a small filter, from which it is dissolved again by hot absolute alcohol, after it has been washed with petroleum ether. Barger and Shaw prefer to place the chloroform solution in a tall 50 c.c. or 100 c.c. stoppered measuring cylinder, in which the digitoxin is precipitated and allowed to settle overnight. The following day the clear liquid, containing impurities, is decanted, and the digitoxin washed

by shaking it with a further quantity of petroleum ether. This is decanted, and finally the digitoxin, mixed with some petroleum ether, is dissolved in hot absolute alcohol. The solution is washed into a weighing bottle and evaporated; dry ether is added and evaporated off, and then the substance, "pure digitoxin Keller," is weighed. Almost two-thirds of Keller's "digitoxin" is really digitoxin.

The results to be obtained from well-prepared tinctures vary from 0.45 to 0.75 per cent of crude digitoxin, the leaves containing from 0.06 to 0.1 per cent as assayed by this method.

It is probable that this method does not give really correct, but only comparative results.

Tincture of Digitalis.—This is an extract of the leaves with 60 per cent alcohol. A genuine tincture should have the following characters:—

Specific gravity	0.930 to 0.935
Solid residue	2.9 „ 3.7 per cent
Alcohol by volume	54 „ 56 „
Digitoxin (as estimated by Keller's process)	0.4 „ 0.75 „

ELATERIUM.

Elaterium, the sedimentary matter from the juice of the fruit of *Ecballium elaterium*, is official in the Pharmacopœia, as well as its active principle elaterin $C_{20}H_{28}O_6$.

The official requirements for elaterium are that it should not give any reactions for carbonates and starch. It should yield 50 per cent to boiling alcohol. When exhausted with chloroform and the solution evaporated, and the residue washed with ether, and the process of solution, evaporation, and washing repeated, at least 20 per cent of elaterin should be so obtained.

The drug occurs as light, thin, pliable pieces, and should not yield more than 14 per cent to 15 per cent of ash. The average composition of the drug is as follows:—

	Per cent
Water	10 to 12
Mineral matter	12 „ 15
Elaterin	22 „ 30
Glucosides	traces
Inert matter	40 to 50

Elaterin is described in the Pharmacopœia as being almost insoluble in water, sparingly soluble in 90 per cent alcohol, but readily soluble in chloroform. It is neutral to litmus; with melted phenol it yields a solution which gives a crimson colour, rapidly changing to scarlet, on the addition of sulphuric acid. It is not precipitated by tannic acid, mercuric chloride, or platinum chloride solutions.

Pure elaterin melts at about 225° to 230°, but the commercial product which fulfils the requirements of the Pharmacopœia, melts at from 208° to 215°. It is highly lævorotary, having a specific rotation of about -42° in chloroform solution.

Power and Moore ("Pharm. Jour." 1909, **83**, 501) state that an

English-made elaterium gave 5·3 per cent of moisture, 6·7 per cent of mineral matter, and an inert aqueous extract of 6 per cent. The dried insoluble matter gave 57 per cent of matter soluble in chloroform and alcohol used successively, the insoluble matter being quite inert. The chloroform and alcohol extracts (continued) gave the following extracts :—

- (1) With petroleum ether, 15 per cent.
- (2) With ether, 73 per cent consisting chiefly of elaterin (crystalline) melting at 217° to 220°.

This latter result indicates 30 per cent of elaterin in the elaterium. The crude drug contains in addition to this amount of elaterin, a considerable proportion of an inert crystalline compound melting at about 230° with decomposition, and having a specific rotation of over -50° .

ERGOT.

Ergot, the sclerotium of *Claviceps purpurea*, a fungus whose spores have developed in the ovary of *Secale cereale*, known usually as "ergot of rye," is official in the Pharmacopœia. No standard is given for it, nor for its three official preparations, extract of ergot; liquid extract of ergot; and ammoniated tincture of ergot.

Numerous constituents of ergot have been described, but it appears that many of them are impure; the well-defined bodies to which the drug owes its activity are two alkaloids cornutine (ergotoxine) $C_{35}H_{41}N_5O_6$ and ergotinine $C_{35}H_{39}N_5O_5$.

An acid which has probably not yet been separated in a state of purity exists which is termed ergotinic acid (sclerotic acid?). For details of the indefinite and uncertain compounds reported upon, reference may be made to the following papers: Tanret ("Jour. Pharm. Chim." 6, 24, 397); Barger and Carr ("Pharm. Jour." 4, 23, 257), and Jacoby ("Chem. Central." 1897, 483 and 1059).

Ergot should contain from 3 per cent to 6 per cent of mineral matter. Various methods have been proposed for its assay, but most authorities hold the opinion that it must be physiologically standardized.

Keller ("Apoth. Zeit." 22, 183) gives the following process for estimating the active principle which he terms ergotin :—

Twenty-five grms. of the powdered ergot is freed from fat by extraction with petroleum ether, and then treated with 100 c.c. of ether; 20 c.c. of water is added after an hour, and 1 gm. of magnesia, and then well shaken for an hour. After standing, 80 c.c. of the ether is separated, corresponding to 20 grms. of ergot, and this is extracted with dilute hydrochloric acid. The acid extraction is repeated several times, the mixed acid liquids are then rendered alkaline, and extracted with ether. The ether extraction is repeated three times, and the mixed ethereal liquids are evaporated in a tared basin, and the residue, consisting of fairly pure ergotin, is weighed.

Liquid Extract of Ergot is an aqueous extract of the drug, preserved by means of alcohol. A pure extract should have the following characters :—

Specific gravity . . .	1.005 to 1.025
Solid residue . . .	12 „ 15 grms. per 100 c.c.
Alcohol by volume . . .	30 „ 32 per cent

Ammoniated Tincture of Ergot.—This is an extract of the drug made by means of a mixture of ammonia and alcohol. A genuine preparation should have the following characters:—

Specific gravity . . .	0.935 to 0.942
Solid residue . . .	2.8 „ 4 grms. per 100 c.c.
Alcohol by volume . . .	50 „ 52 per cent

On adding solution of caustic soda and distilling the liquid through a well-cooled condenser, about 1 per cent of NH_3 should be obtained. The precautions necessary in determining the alcohol are the same as in the case of ammoniated tincture of guaiacum (p. 458).

Wood ("Amer. J. Pharm." 1909, **81**, 215) claims that the therapeutic activity, as determined physiologically, of extract of ergot is in almost direct ratio to the amount of resin precipitated by water. He recommends the estimation of this resin by diluting the liquid extract with twice its volume of water and repeatedly extracting with benzene, evaporating the solvent and drying the resin on a water bath. A small amount of another active principle is *not* extracted by benzene, but this amounts to only a small proportion of the whole of the active principles. The proportion of resin so extracted should vary from 0.48 per cent to 0.68 per cent of the liquid extract.

It is to be noted, however, that Tanret has recently isolated a sulphur-containing base, ergothionine, $\text{C}_9\text{H}_{15}\text{O}_2\text{N}_3\text{S}$, which is soluble in water, which is possibly therapeutically active; and Carr and Barger ("Journ. Chem. Soc. Trans." 1907, **91**, 337) and Barger ("Journ. Chem. Soc." 1909, 1123) have isolated soluble alkaloids (ergotoxin and p-hydroxyphenylamine) which are certainly physiologically active substances.

GELSEMIUM.

The root and rhizome of *Gelsemium nitidum* are official in the British Pharmacopœia. No standards are given for the drug.

It contains an alkaloid gelsemine, which probably has the formula $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_2$ (or according to Sayre $\text{C}_{14}\text{H}_{15}\text{NO}$). It is a crystalline base possessing unknown therapeutic activity. Traces of gelseminine are also present, a powerfully toxic alkaloid which occurs to the extent of about 0.5 per cent in the drug. It is therefore obvious that a determination of the alkaloidal value of the drug or its preparations gives little information as to the real value of the substance. Scopoletin is also present in small amount.

Gelsemium root yields from 2 per cent to 4 per cent of ash, rarely up to 5 per cent.

It contains from 0.3 per cent to 0.9 per cent of alkaloids as determined by the process described under tincture of gelsemium.

Tincture of Gelsemium.—This is the extract from two ounces of the drug with 60 per cent alcohol to make one pint of tincture. It

should have the following characters (none of which are mentioned in the Pharmacopœia) :—

Specific gravity	0.920 to 0.928	
Solid residue	1.20 „ 1.30	grms. per 100 c.c.
Alcohol (by volume) . . .	56 „ 57.5	per cent
Alkaloids	0.02 „ 0.03	„

The alkaloids may be determined as follows :—

Two hundred c.c. of the tincture (if so much is available) are evaporated to a thick liquid, rendered alkaline and extracted with chloroform three times. The chloroform is then extracted with slightly acidulated water, the aqueous liquid rendered alkaline and the alkaloid again dissolved out with chloroform. The chloroform is washed with water until the water ceases to give a pink colour with phenol-phthalein and then evaporated in a thin porcelain dish and the alkaloid weighed. It is preferable, however, to titrate it, by adding a calculated excess of one-twentieth normal hydrochloric acid and titrating back with one-twentieth normal solution of barium hydrate, using methyl orange or iodeosin as indicator. Each c.c. of one-twentieth normal acid is equivalent to 0.0161 grm. of gelsemine, taking $C_{20}H_{22}N_2O_2$ as the formula.

Gelsemine must be distinguished from the resinoid substance to which the same name has been applied and which is a powdered alcoholic extract of the root. Gelsemine melts at 178° and crystallizes from acetone in needles. If a minute fragment be allowed to stand with a drop of nitric acid, which is allowed to evaporate spontaneously, a permanent blue-green colour is produced. If a fragment of gelsemine be treated with sulphuric acid and an oxidizing agent, it behaves much like strychnine, except that the colour produced is of a reddish-purple soon changing to blue or red-blue. Gelsemine forms crystalline salts of which the hydrochloride is the only one found in commerce. It is a combination of 1 molecule of alkaloid and 1 of acid.

HYDRASTIS.

The dried roots and rhizome of *Hydrastis canadensis* are official in the Pharmacopœia, but no standards are given. A liquid extract and a tincture of the drug are also official.

The principal constituent of the drug is the alkaloid hydrastine $C_{21}H_{21}NO_6$ (see below), together with some berberine $C_{20}H_{17}NO_4$ and canadine. The first-named alkaloid is present to the extent of 1.5 per cent to 4 per cent, berberine to the extent of about 3 per cent, and canadine only in traces.

This drug yields from 4 per cent to 10 per cent of ash on incineration.

The drug should be assayed for its alkaloidal value, it being necessary to separate the hydrastine from the berberine. The best method for this determination is Maben's ("Year-book of Pharmacy," 1901, 408). Ten grms. of finely powdered hydrastis are extracted with hot 90 per cent alcohol in a Soxhlet tube. The liquid, containing the whole of the extractive, is made up, when cool, to 100 c.c.

Place 25 c.c. of the above extract in a wide-mouthed flask of about 8 ounce capacity ; add $1\frac{1}{2}$ c.c. of hydrochloric acid (32 per cent), $\frac{1}{4}$ c.c. of concentrated sulphuric acid, and 125 c.c. of ether. Cool, shake well and allow the mixture to stand twenty-four hours in a refrigerator, and the crystals of berberine hydrochloride will separate. Filter through a tared paper and preserve the filtrate. Wash the crystals with a mixture of equal volumes of alcohol and ether until the washings cease to give an acid reaction. Add the washings to the filtrate preserved as above directed. Dry the crystals at 105° C., and weigh. The result multiplied by 0.9017 gives the berberine. This multiplied by 4 is equivalent to the berberine in 10 grms. of the drug.

Render the combined filtrate and washings from the berberine neutral or only faintly acid. Evaporate nearly to dryness on the water bath ; treat the residue with hot water in small quantities, filtering same into a stoppered separating funnel until the washings from the residue cease to give an alkaloidal reaction with the ordinary reagents. (The extraction of the alkaloid from the resinous mass left after the evaporation of the combined filtrate and washings may be somewhat expedited at this point by the addition of a few drops of alcohol at each extraction with water, evaporating off the alcohol each time before the aqueous washing is poured off.) Add to the aqueous extract in the separating funnel ammonia water to render alkaline, and extract the hydrastine by agitation with ether. Continue the extraction with ether until the hydrastine is entirely removed ; evaporate off the excess of ether, and re-extract the hydrastine by means of several portions of 5 per cent sulphuric acid, and from the combined acid washings extract the hydrastine again by shaking with several portions of ether, after having rendered the solution alkaline with ammonia. Finally evaporate off the ether, dissolve the hydrastine in an excess of $\frac{N}{20}$ acid, titrating back the excess with $\frac{N}{100}$ alkali in the usual manner, using cochineal as an indicator. Each c.c. of alkali is equal to 0.00383 grm. of hydrastine and this multiplied by 4, gives the hydrastine in 10 grms. of the drug.

Gordin and A. B. Prescott ("Amer. Journ. Pharm." 1899, 518-22) recommend the following process of assay. Ten grms. of the powdered root are stirred into a paste with a mixture of alcohol, concentrated ammonia, and ether (1 : 1 : 6 parts by volume), and allowed to remain in a closed vessel for several hours. The mixture is then dried, at first in a current of air, and then over sulphuric acid under diminished pressure ; the residue is transferred to a Soxhlet apparatus, being rinsed out with powdered barium nitrate, and the hydrastine is extracted completely with absolute ether ; the ether is evaporated from the extract, and the residue dissolved in acidified water, and the solution diluted to 100 c.c. In a graduated 100 c.c. flask, 20 c.c. to 30 c.c. of a standard iodine solution (of about 1 per cent strength) are placed, 20 c.c. of the filtered hydrastine solution run in, and the mixture is diluted to the mark and shaken until the pentiodide has all separated ; the mixture is then filtered, and the excess of iodine deter-

mined by titrating 50 c.c. of the filtrate with standard sodium thiosulphate solution. Every 1 part of iodine used corresponds with 0.607 part of hydrastine. Or the alkaloid may be estimated gravimetrically by shaking 20 c.c. of the filtered hydrastine solution with petroleum ether and ammonia, removing the alkaloid from the petroleum ether solution by shaking with acidified water, and then from the acid solution with ammonia and ether; the ethereal solution is finally evaporated at the ordinary temperature, and the residue of hydrastine weighed.

The residue in the Soxhlet apparatus contains the berberine, which is not soluble in absolute ether; it is dried by passing a current of dry air through the apparatus, and is then extracted with alcohol. The alcohol is removed from the extract by heating it with 200 c.c. of water on the water bath; the residual liquid is acidified with acetic acid, cooled, and filtered into a conical flask; in this it is shaken for ten minutes to fifteen minutes with 6 c.c. to 8 c.c. of acetone, and enough 10 per cent caustic soda solution to render it alkaline, and set aside for two hours to three hours. The precipitated acetone compound is washed, and warmed in the same flask with 200 c.c. to 300 c.c. of very dilute sulphuric acid until it has all dissolved, the solution is poured into a long-necked Kjeldahl flask and boiled for one and a half hours to two hours; when cold, it is added to 100 c.c. of $\frac{N}{20}$ potassium iodide solution contained in a graduated 1000 c.c. flask, diluted to the mark, shaken, and left overnight. Then 500 c.c. are filtered from the precipitate of berberine hydriodide into another 1000 c.c. flask, treated with 50 c.c. $\frac{N}{20}$ silver nitrate and nitric acid, diluted to the mark, and filtered; the excess of silver is determined by titrating 500 c.c. of the filtrate with $\frac{N}{40}$ ammonium thiocyanate. The number of c.c. of the iodide solution used, multiplied by 0.167125, gives the percentage of berberine in the root.

Schreiber's process gives good results. It is as follows ("Pharm. Post," 36, 321): 10 grms. of the powdered root are dried on the water bath, the moisture being thus determined. The dry residue is moistened with a mixture of ammonia, 5 c.c., alcohol, 5 c.c., and ether, 30 c.c., and dried. It is then extracted in a Soxhlet with ether; and the ether extract shaken with 15 grms. of 5 per cent hydrochloric acid in a graduated cylinder. The ethereal layer is decanted, the acid extract washed with more ether to remove resinous matter, and the ether decanted. The volume of ether over the acid liquor is then adjusted to exactly 50 c.c. Ten c.c. of ammonia are added and the whole well shaken until all the precipitated alkaloid is dissolved in the ethereal layer. After separation, 40 c.c. of this is decanted ($= \frac{4}{5}$ of the whole), into a tared capsule, about half the ether evaporated off at a gentle heat, the rest allowed to evaporate spontaneously. In this manner almost colourless crystals of hydrastine are obtained which are finally dried to constant weight on the water bath.

Matthes and Rammstedt ("Archiv der Pharm." 245, 112) have recommended picronic acid (dinitro-phenyl-methyl-pyrazolone) as a precipitant of hydrastine—and also for the pilocarpine and the mixed alkaloids of nux vomica. The process described is interesting, but it is neither so accurate nor so simple as the above, and therefore for details the original paper should be consulted.

Liquid Extract of Hydrastis.—This preparation is a 45 per cent alcohol extract of the drug of such strength that 1 fluid ounce contains the active principles of one ounce of the drug. No official standards exist. When properly prepared it should have the following characters:—

Specific gravity	1.025 to 1.040
Solid residue	20 " 24 grms. per 100 c.c.
Alcohol by volume	36 " 40 per cent
Total alkaloids	4 " 6 gr. per 100 c.c.
Hydrastine	1.5 " 3 "

Tincture of Hydrastis is an extract with 60 per cent alcohol of one-tenth the strength of the liquid extract. No standards are official. It should have the following characters:—

Specific gravity	0.923 to 0.929
Solid residue	2 " 2.5 grms. per 100 c.c.
Alcohol by volume	56 " 58 per cent
Total alkaloids	0.4 " 0.6 gr. per 100 c.c.
Hydrastine	0.15 " 0.3 "

The alkaloids in the above two preparations should be assayed in the same manner as in the root. In the liquid extract 10 c.c. may be used, and for the tincture 100 c.c.

Hydrastine $C_{21}H_{21}NO_6$ forms white prisms soluble in 120 parts of 90 per cent alcohol, and in two parts of chloroform. When pure it melts at 132° . The approximate purity of the alkaloid should be checked by titrating it with one-twentieth normal hydrochloric acid. One c.c. of $\frac{N}{20}$ HCl is equivalent to 0.01916 grm. of hydrastine,

using methyl orange or cochineal as indicator. A solution in dilute sulphuric acid is rendered fluorescent (blue) by the addition of a trace of potassium permanganate. The alkaloid should not be colored red on the addition of chlorine water—berberine gives a strong red colour. A solution of hydrastine (neutral) is precipitated by potassium bichromate solution, and if the separated precipitate be touched with H_2SO_4 it instantly becomes bright red—the colour fading in a few seconds.

Berberine $C_{20}H_{17}NO_4$ exists also in *Berberis vulgaris* and other plants. The alkaloid crystallizes with 4 molecules to 6 molecules of water of which the equivalent of 2.5 molecules remain after heating to 100° . It forms yellow silky needles melting at 145° and decomposing at 150° . It is only slightly soluble in cold water, almost insoluble in ether, but readily soluble in hot alcohol. An aqueous solution is coloured blood-red with chlorine water. If a trace of berberine be boiled with hydriodic acid, the liquid diluted with water

and rendered slightly alkaline with ammonia, an intense blackish-violet colour is produced.

Berberine hydrochloride, $C_{20}H_{17}NO_4 \cdot HCl$, $2H_2O$ and berberine phosphate $C_{20}H_{17}NO_4 \cdot 2H_3PO_4 \cdot 2H_2O$, are the commoner salts of the alkaloid. They should give the reactions described under berberine and yield the amount of alkaloid indicated by the above formula, when dissolved in water, the liquid rendered alkaline with ammonia and extracted with warm amyl alcohol.

HYOSCYAMUS.

Hyoseyamus leaves are official in the Pharmacopœia, being described as the fresh leaves and flowers with the branches to which they are attached, of *Hyoscyamus niger*; also the leaves and flowering tops separated from the branches and carefully dried, collected from the flowering biennial plants. No standards are given.

From the fresh leaves, etc., an official green extract is prepared, whilst from the dried leaves and flowering tops an official tincture is prepared.

The principal constituent of the leaves is the alkaloid hyoscyamine (p. 521), together with smaller quantities of atropine and hyoscyne. The alkaloidal value of the dried leaves varies from 0.05 per cent to 0.18 per cent, rarely up to 0.25 per cent.

The mineral matter varies from 9 per cent to 14 per cent.

A microscopic examination of powdered hyoscyamus leaves (henbane leaves) shows a marked difference between these leaves and those of stramonium and belladonna. In henbane leaves the mesophyll is heterogeneous and asymmetrical, the cells of the spongy parenchyma often containing prismatic crystals of calcium oxalate; whereas stramonium and belladonna leaves contain chiefly cluster crystals and sandy crystals respectively (Greenish).

According to Greenish the diagnostic characters of this drug are:—

- (1) Characteristic glandular hairs.
- (2) Prismatic crystals of calcium oxalate.
- (3) Epidermal cells with wavy walls.
- (4) Stomata surrounded by three or four cells of which one is larger than the other.
- (5) The absence of pericyclic fibres.

The sketch on page 556 represents the powdered leaves.

The alkaloids in the leaves may be estimated by the process used for the tincture.

Tincture of Hyoscyamus.—This is made by extracting 2 ounces of leaves with sufficient 45 per cent alcohol to produce 1 pint of tincture. No official standards exist, but a genuine tincture should have the following characters:—

Specific gravity	0.950 to 0.960
Solid residue	2.3 „ 3.6 grms. per 100 c.c.
Alcohol by volume	43 „ 44 per cent
Alkaloids	0.008 „ 0.015 „

The alkaloids may be determined in exactly the same manner as that described on p. 603 for tincture of stramonium.

Green Extract of Hyoscyamus.—No standard exists for this preparation, but properly prepared samples will contain from 0.2 to 0.4

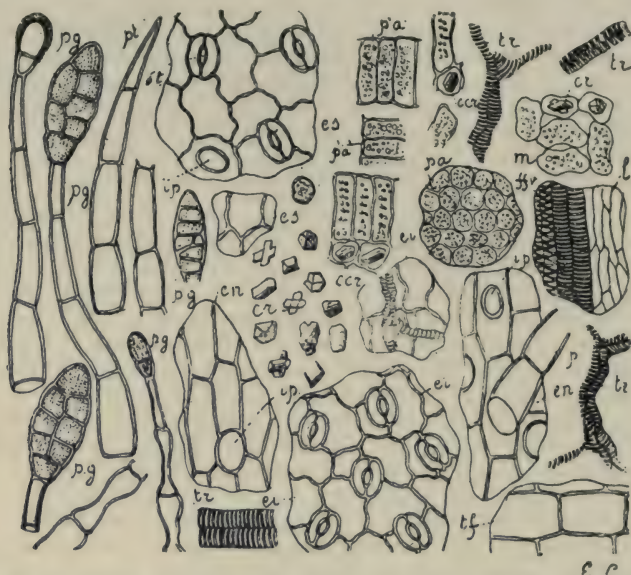


FIG. 51.—Powdered henbane leaves $\times 240$. *ccr*, crystal cells; *cr*, crystals of calcium oxalate; *ei*, lower epidermis; *es*, upper epidermis; *ffv*, portion of fibrovascular bundle of midrib; *ip*, scar of fallen hair; *m*, spongy parenchyma; *pa*, *p'a'*, palisade cells; *pg*, glandular hairs; *pt*, simple hairs; *st*, stomata; *tf*, cortical parenchyma of midrib; *tr*, tracheids and vessels. (Greenish & Collin.)

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per cent of alkaloids, with an average value of 0.3 per cent, when determined in the manner described on p. 518 for green extract of belladonna.

IPECACUANHA.

Ipecacuanha root is the dried root of *Psychotria ipecacuanha* (*Cephalis ipecacuanha*) and is thus described in the British Pharmacopœia :—

"Ipecacuanha occurs in somewhat tortuous pieces not often exceeding six inches in length, and one quarter of an inch in thickness. It varies in colour from dark brick-red to very dark brown and is closely annulated externally, the annulations not taking the form of narrow merging ridges (distinction from *Carthagenia ipecacuanha*). It breaks with a short fracture, the fractured surface exhibiting a thick greyish cortex, which usually has a resinous but sometimes a starchy appearance and a small dense central portion. When examined under the microscope the cortex exhibits small compound starch grains and

raphides; the wood contains no vessels. The odour is slight, the taste bitter."

Ipecacuanha occurs on the markets in its natural state, but in the retail shops is frequently purchased in the form of powder. In the former case it is liable to be mixed with roots of similar appearance, as well as with the stems of the genuine plant, whilst in the state of powder, other adulterants may be present.

The following description of the commercial root is due to E. M. Holmes:—

The ipecacuanhas of English commerce may be divided into two sections:—

1. Those that are derived from the genus *Cephalis*.

2. Those that are derived from other genera belonging to the same or to different natural orders.

1. OFFICIAL IPECACUANHA (*Cephalis ipecacuanha*, Rich).—Of this kind there are several commercial varieties or qualities.

A. *Brazilian or Rio Ipecacuanha*.—When of good quality the roots are one or two lines in diameter, and externally of a reddish or blackish-brown colour. Specimens without a powdery surface are to be preferred, since the powdery appearance is often due to the remains of moulds. A good sample should yield about 80 per cent of bark.

B. *Indian Ipecacuanha*.—This is derived from the plant cultivated in Johore (Straits Settlements), and has only been introduced during recent years. It is imported from Singapore. Commercially it is distinguished from the Brazilian kind by the presence of the delicate rootlets, which usually occur to a much smaller extent in the South American drug. According to an analysis by Ransom, it contains 1·7 of (the so-called) emetine as against an average of 1·66 per cent in the Brazilian kind, and may therefore be supposed to be of good quality.

C. *Mouldy Ipecacuanha*.—It is calculated that about three out of every four serons of ipecacuanha root imported have been damaged by sea-water during the voyage to Europe or during transit to the coast from the place of collection ("Pharmacographia," 2nd ed., p. 375). It has been maintained by some that the mouldiness does not affect the amount of alkaloid present. This statement needs confirmation.

D. *Woody Ipecacuanha*.—It is of the prevalence of this quality in commerce that complaints have recently been made. It is characterized by the presence of an unusual amount of stem. A small piece of the woody stem is often attached to the root in good samples, but in woody ipecacuanha it may amount to 30 or 50 per cent of the whole. The stem is easily recognized by its smooth not annulated surface, remarkably thin bark, and by the presence, visible under a good lens, of pith in the centre of the woody column. As the stem is not official in the Pharmacopœia, and is probably one-third weaker than the root, it should not be used for pharmacopœial preparations.

E. *Doctored Ipecacuanha*.—This quality consists of inferior, woody or mouldy ipecacuanha that has been washed and dried. It has a

dark colour and clean epidermis, contains few large pieces, and the bark has been much broken off the root in the process of washing. By this latter character and its dark colour it is easily recognized.

2. CARTHAGENA OR SAVANILLA IPECACUANHA (*Cephalis acuminata*, Karsten).—This kind of ipecacuanha has recently been imported in increasing quantities. It is, however, by no means a new article in commerce. It is probably identical with the grey annulated ipecacuanha of Pereira, which he describes as "occurring in pieces of larger diameter than in ordinary ipecacuanha, with fewer, more irregular and less prominent rings". Professor Guibourt remarked that considerable quantities of it arrived unmixed with the ordinary sorts, and that he thought it to be a distinct kind coming from a different part of Brazil, and derived from another species of *Cephalis* (Pereira, "Mat. Med." Vol. II, pt. 2, p. 58). This description is exactly applicable to the Carthagena ipecacuanha of the present day, which is characterized by the less prominent and more distant rings and transverse fissures.

Under the microscope it presents, according to Karsten, a distinctive feature in the fact that the cortical parenchyma forms two distinct layers, which is not the case in ordinary ipecacuanha. The radiate structure of the central woody column is also more distinctly visible than in the ordinary ipecacuanha.

Carthagena ipecacuanha has been analysed by Dr. Wimmel, Conroy, and others, and the results obtained indicate that it varies like the Brazilian drug, in percentage of alkaloids, but that on the whole it is probably not inferior to it in the amount of alkaloid present. It must be remembered, however, that it contains a different crystalline alkaloid which is not chemically identical with that of the Brazilian drug.

SPURIOUS IPECACUANHAS.

Owing to the name "Poaya" being used in a generic sense in South American countries for roots possessing emetic properties, various drugs bearing this name are sent to this country by merchants at intervals of a few years. None of them approach ipecacuanha in therapeutic value. Hence a description of their appearance in the crude state may prove useful.

The plants from which these Poayas are derived belong chiefly to the natural orders *Rubiaceæ* and *Violaceæ*, and one to the *Polygalaceæ*. Those which have been identified in English commerce are three in number, viz.: (1) *Psychotria emetica*, (2) *Richardsonia scabra*, and (3) *Ionidium ipecacuanha*.

Several other spurious ipecacuanhas, more or less resembling these three, have at intervals been imported into Europe, but probably have not been distinguished from them, except in one or two cases in which a microscopic examination has been made. Of these I only propose to notice those that have been met with in commerce in this country.

A. BLACK OR GREATER STRIATED IPECACUANHA (*Psychotria emetica*, Mutis).—This is so called from its black epidermis. The root is slightly larger than Rio ipecacuanha and strongly constricted at inter-

vals of about an inch, more or less, the intermediate portions being cylindrical and striated longitudinally. Internally the cortical portion is thick in proportion to the woody column, and presents a horny appearance, and sometimes a purplish tint. A decoction of the root gives evidence of the presence of a reducing sugar, but not of starch. According to Ransom it contains traces (.016 per cent) of emetine, or of an alkaloid giving the same reactions. The woody column is dense, and not visibly porous.

B. LESSER STRIATED IPECACUANHA (*Richardsonia species*).—This drug externally has also a black colour and striated appearance, and constrictions at intervals like the greater striated ipecacuanha, but it presents marked differences internally. The cortical portion is often of a dark violet tint, and is full of starch, which can readily be detected in a cold decoction by iodine, and the woody column is seen to be distinctly porous when viewed under an ordinary lens. Professor Planchon refers it provisionally to the genus *Richardsonia*. Examined by Ransom it was found to contain .027 per cent of emetine.

C. UNDULATED IPECACUANHA (*Richardsonia scabra*).—Externally the root is of a greyish-brown colour, and differs from ipecacuanha in not having raised rings. It is, however, marked with deep constrictions, often on alternate sides, which gives the root a somewhat undulated or falsely annulated appearance. In transverse section the root is seen to be white, and starchy, and sometimes has a faint violet tint, and the woody column is yellow and porous. It has been stated to contain emetine, but the statement needs confirmation.

D. (1) WHITE IPECACUANHA (*Ionidium Ipecacuanha*).—This drug differs from the foregoing in its pale yellowish-brown colour and much-branched character. The woody column is large, yellow, and porous, and the cortical portion is thin, so that the root is more woody in character than *Richardsonia*, but it has transverse fissures and constrictions like the latter. It does not contain starch.

D. (2) A root, supposed to be that of *Ionidium ipecacuanha*, entered the London market in 1884, and was examined by W. Kirkby, who pointed out that it differed from the root of that plant in having large wedge-shaped groups of sclerenchymatous cells in the cortical portion, and more or less broad medullary rays in the woody column ("Pharm. Journ." 3, xvi. 126).

E. FALSE INDIAN IPECACUANHA.—Some years ago a quantity of a small root said to be imported from Southern India, was offered in the London market as ipecacuanha. It differs from true ipecacuanha in colour, which is of a pale reddish-brown, but it presents a ringed appearance.

The following key to the microscopical structure of the commercial ipecacuans may perhaps prove useful. It is based upon a paper on this subject by Tschirch and Ludtke in the "Archiv der Pharmacie," 1883, p 441.

I. Woody column containing chiefly tracheids, but no vessels.

A. Root-bark containing starch and raphides.

1. Parenchyma of bark uniform = *Rio Ipecacuanha*.

2. Parenchyma of bark forming two layers = *Carthagenia Ipecacuanha*.

B. Root-bark containing no starch, but sugar.

Woody centre not visibly porous = *Greater Striated Ipecacuanha*.

II. Woody cylinder containing vessels, wood-cells, and medullary rays.

A. Root-bark, containing starch.

1. Medullary rays composed of a single row of cells, woody centre visibly porous = *Lesser Striated Ipecacuanha*.

2. Medullary rays forming two or three rows of cells = *Undulated Ipecacuanha*.

B. Root-bark containing inulin.

1. Medullary rays of a single row of cells, no starch, sphæraphides in the bark = *White Ipecacuanha (a)*.

2. Bark contains stone cells.

3. Medullary rays broad = *White Ipecacuanha (b)*.

III. Rhizome having a monocotyledonous structure, brown pigment cells in parenchyma, acicular raphides and starch present = *False Indian Ipecacuanha*.

It is obvious that the microscopic examination of this drug is a matter of extreme importance. The commonest adulterant of the powdered root is *Carthagenia ipecacuanha*, which is not official in the Pharmacopœia, as it contains the characteristic alkaloids, emetine and cephaeline in quite different proportions to those found in the Rio root.

Samples of powder should be compared microscopically with type powders from the two roots. It will be found that the starch grains from Rio root are about half as large as those in *Carthagenia* root, but sometimes the larger grains of Rio root are equal in size to the smallest of *Carthagenia* root.

The powder should show an absence of vessels, but there are to be found perforated tracheids, and acicular raphides. The presence of stem in the sample is revealed by the sclerenchymatous cells, lignified pith cells and spiral vessels.

The principal constituents of *ipecacuanha* root are two alkaloids, emetine and cephaeline, which also occur in *Carthagenia* root in which the cephaeline predominates over the emetine, whereas in Brazilian root the reverse is the case.

The chemistry of these bodies has received much attention from Paul and Cownley, who give an interesting account of the matter in the "Pharmaceutical Journal" (3, xxv. 111, 373, 690).

According to these chemists emetine is a practically colourless amorphous alkaloid of the formula $C_{15}H_{22}NO_2$ (Kunz-Krause considers the formula to be $C_{30}H_{40}N_2O_5$;—it is probable that the formula of Paul and Cownley requires doubling). It melts at about 68° , and is readily soluble in ether, alcohol, and chloroform but insoluble in alkalis. It forms salts, containing one equivalent of acid, and easily soluble in water, but not easily crystallizable. Cephaeline has the formula $C_{14}H_{20}NO_2$ (or $C_{28}H_{40}N_2O_4$), and is a crystallizable alkaloid melting at about 102° . It is less soluble in ether than emetine, but

it is freely soluble in alcohol and chloroform. It is much more soluble in warm petroleum ether than emetine, and is easily soluble in alkaline solutions. It forms well-defined neutral salts, which crystallize from acid solutions.

A third alkaloid exists in ipecacuanha root, melting at 138° and forming lemon-yellow prisms. It is present, however, in very small amount, and is known as psychotrine. Apart from these alkaloids, ipecacuanha contains numerous other substances, the following being the composition of a root most exhaustively examined by Cripps and Whitby:—

	Per cent
Moisture	10.85
Volatile oil	trace
Free fatty acid	0.16
Neutral fat	0.11
Wax (?)	0.03
Acid resins soluble in ether	0.05
Indifferent resins	0.23
Substance allied to quercitrin	0.03
Tannin (total)	1.13
Phlobaphene	0.34
Saccharose	2.12
Dextrose (total)	4.06
Dextrine	2.08
Mucilage	3.81
Albumen precipitated by boiling	3.10
Albumen not precipitated by boiling	0.23
Albumen, pectin, etc., insoluble in H_2O	3.34
Albumen not precipitated by alcohol	3.12
Organic acids and allied bodies	1.48
Alkaloid removed by ether from alcoholic extract	1.91
Alkaloid removed by chloroform	0.24
Alkaloid, etc., removed by chloroform from acid solution	0.10
Alkaloid from aqueous extract	0.17
Colouring matter and decomposition products	2.52
Resinous (?) matter not removed by agitation with ether, etc.	0.07
Starch	44.44
Cellulose, lignin, etc.	11.30
Ash, soluble in H_2O	0.53
Ash, soluble in HCl	1.69
Ash, insoluble in HCl	0.21
	99.65

Arndt ("Year-book of Pharmacy," 1889, 136) claims to have isolated 0.3 per cent to 0.5 per cent of choline from the root, but this is denied by Cripps.

The ash of ipecacuanha is rarely above 4 per cent, any excess being usually due to residual earthy matter left on the roots. On page 562 are figures for pure samples of Rio and Carthagena roots obtained by the author.

The Assay of Ipecacuanha Alkaloids.—The official process of the British Pharmacopœia for the determination of alkaloids in the liquid extract, may be applied to the root, using 20 grms. for the determination. The root in coarse powder should be exhausted with 90 per cent alcohol in a small percolator, and when exhausted, the mass should be mixed with 2 grms. of pure lime, and allowed to stand for

	Moisture.	Ash.	Ash Soluble in H ₂ O.	Ash Insoluble in HCl.
Rio root . . .	10.42	2.00	0.49	0.24
" " . . .	11.55	3.25	0.58	0.38
" " . . .	11.90	3.90	0.52	0.69
" " . . .	10.80	4.35	0.60	0.60
Carthageua root . .	10.25	4.65	0.62	0.75
" " . . .	11.80	3.98	0.59	0.89
" " . . .	12.05	5.01	0.62	0.91



FIG. 52.—Powdered ipecacuanha.

twenty-four hours, and then again exhausted with 90 per cent alcohol and the percolates mixed. The liquid should be concentrated to about 20 c.c., and diluted with 20 c.c. of water. The alcohol is then evaporated on the water bath, and to the warm solution a slight excess of solution of subacetate of lead is added. The liquid is filtered, and the precipitate well washed. Excess of lead is removed from the filtrate by dilute sulphuric acid. The liquid is again filtered, the precipitate washed and the liquid transferred to a separator. Excess of ammonia is added, and the liquid well shaken with 25 c.c. of chloroform. The chloroform is separated, and the extraction re-

peated twice. The mixed chloroform solutions are evaporated, and the residue dried at 80° C., and weighed.

There is always a slight loss by this process, as alkaloids are precipitated with the lead, but it does not exceed 0.1 per cent.

Keller prefers the following process which gives very concordant results: 10 grms. of the dried and finely powdered root are well agitated in a bottle of 150 c.c. capacity with 40 grms. of chloroform and 60 grms. of ether; 10 grms. of solution of ammonia are then added, and the agitation repeated at frequent intervals during one hour, after which another 5 grms. of solution of ammonia are added, and again well agitated with the mixture. After settling, 50 grms. of the decanted solution, representing 5 grms. of the dried root, are carefully distilled in a weighed Erlenmeyer flask; the varnish-like residue is twice treated with 10 c.c. of ether, and evaporated by forcing a current of air through the flask. After the last traces of ether have been removed, the residue is dried in a water bath and weighed. For the titration of the alkaloid it is dissolved in 10 c.c. of absolute alcohol with the aid of heat, sufficient water added to produce a permanent turbidity, and the titration then carried out with decinormal hydrochloric acid in the presence of a few drops of hæmatoxylin solution as an indicator. Each c.c. of the decinormal acid represents 0.0254 gm. of emetine. An improvement of this assay consists in the removal of the fat from the ipecacuanha root by percolation with ether previous to the process described. This preliminary treatment renders the subsequent titration more easy and distinct.

Kottmayer ("Phar. Post." 1892, 913, and 933) claims that the following process gives the most accurate results, and according to the author's experience this is true, but it is far too tedious for ordinary use:—

The powdered root should be used without drying, since heating renders the extraction of the alkaloid more difficult. Fifteen grms. of the powdered root are placed in a bottle with 148 c.c. of 90 per cent alcohol and 2 c.c. of hydrochloric acid of specific gravity 1.12, and digested, with frequent agitation, at 40° C. for four days; after cooling to 15° C., 100 c.c. are removed, mixed in a capsule with 20 c.c. of a 10 per cent alcoholic lead acetate solution (50 per cent alcohol), and, after the addition of 1.5 grms. of slaked lime, evaporated, with occasional stirring, to a pasty consistency; 5 grms. of powdered glass are then incorporated, and the heating is continued on a water bath with constant stirring until a dry powder results. This is extracted for ten hours with chloroform, the chloroform solution evaporated in a weighed vessel, dried at 100° C., and weighed. The crude alkaloid thus obtained is dissolved in 2 c.c. of normal hydrochloric acid, the insoluble matter collected on a weighed filter, thoroughly washed, dried, and weighed. The total residue minus the weight of the insoluble resin leaves the weight of the pure alkaloid.

Cripps ("Pharm. Journ." 3, xxv. 1093) recommends the following modification of Lyons' method: 2.5 grms. of the powdered sample are exhausted by a mixture of 250 parts of ether, 10 of ammonia and 20 of alcohol. The alkaloids are separated from the

ethereal solution by repeated extraction with dilute hydrochloric acid, the aqueous solution rendered alkaline with ammonia, and the alkaloid finally dissolved out first by ether and then by chloroform. The solvents are evaporated in a current of air, and the residue weighed after drying at 50° to 60°. This gives the approximate amount, after which it should be dissolved in 5 c.c. of one-twentieth normal hydrochloric, and the excess of acid titrated back with caustic soda. Each c.c. of decinormal acid represents 0.0244 grm. of alkaloids. This figure is based on the average proportions of emetine and cephaeline present. Calculated as emetine the value 0.0254 would be used.

F. C. J. Bird has devised a process for the alkaloidal assay of the root which is both convenient and accurate, and well suited for general work. The drug should be in a fine powder and 10 grms. should be used for the determination. The following are the details of the process. In the event of the chloroform not separating quickly a little ether should be added :—

Rio ipecacuanha in fine powder	10 grms.
Sodium bicarbonate	2 "
Water	5 c.c.

Mix about half the soda with the powdered ipecacuanha, shake the remainder with the water and rub the whole in a small mortar to a uniform moist granular powder.

Amyl alcohol	1 volume	} g.s.
Chloroform	1 "	
Ether	3 volumes	

Add the moistened powder to 20 c.c. of the above solvent, previously placed in D (plugged with cotton wool, as shown on page 565) and macerate for half an hour, with occasional shaking. Force out the liquid by compressing H, and cover the powder with 10 c.c. more menstruum. Agitate vigorously, let stand fifteen minutes and again force out the liquid. Repeat this at intervals of a quarter of an hour until ten or twelve quantities of menstruum have been used or the powder is exhausted.

(If time is of no importance, percolation in the ordinary way may be substituted for the above procedure.)

Agitate the mixed ethereal extracts successively with :—

Dilute sulphuric acid	4 c.c.
Water	6 "
Water	5 "
Water	5 "
Water	5 "
To the mixed acid solutions add carefully ammonium bicarbonate	
	0.5 gm.

Shake out the alkaloid with chloroform containing about one-sixth its volume of ether four times, adding 1 drop of ammonia to the chloroform. Mix the four portions of chloroform and either (1) evaporate, dry below 80° C., and weigh, or (2) dilute with chloroform to 100 c.c. and divide into two equal volumes. Evaporate, dry, and weigh the one

as usual, but remove the chloroform from the other by a current of air and titrate the residue, using the equivalent 0.0244. The titration figure should come within 2 per cent or 3 per cent of the weight.

For the official process of assay of the liquid extract Bird proceeds as follows, the apparatus devised by him greatly expediting the process:—

Liquid extract of ipecacuanha, 20 c.c.; distilled water, 20 c.c.; acetic acid, *q.s.* to faint acid reaction. Evaporate off the alcohol and add distilled water, 20 c.c. liq. plumbi subacet., 10 c.c. Keep the mixture on the water bath for a few minutes until the magma which at first forms changes to a thin liquid, and the precipitate assumes a finely granular condition. Transfer to filter B, and connect A with a water pump. (In the absence of the latter exhaustion of the air by forcibly sucking with the mouth at A, retaining the vacuum by the clip C, ensures a very fair rate of filtration.) Wash the nearly dry solid cake remaining on the filter with distilled water, 30 c.c., added in small portions. To the filtrate in C add acid sulph. dil., 25 c.c.; change the filter paper on B; transfer B to another filter flask, and pour upon it the liquid in C, aiding filtration by a vacuum as before. Wash the cake of lead sulphate with distilled water, 15 c.c. To the filtrate in the same flask add chloroform and 5 c.c. ammonia. Cork the flask, agitate vigorously and transfer contents to D. Connect D with H by the rubber cork G, and having inflated the pressure-ball H, force the chloroform and a portion of the aqueous liquid in D through the filtering medium shown, into E. Draw off the chloroformic layer, which should be perfectly clear and bright, into the tared glass dish F. Return the aqueous liquid in E and D to the filter flask, add chloroform, 25 c.c.; and proceed as before. Repeat a third time with chloroform 25 c.c. Finally collect the three chloroformic layers in F. Evaporate and dry the residue below 80° C.

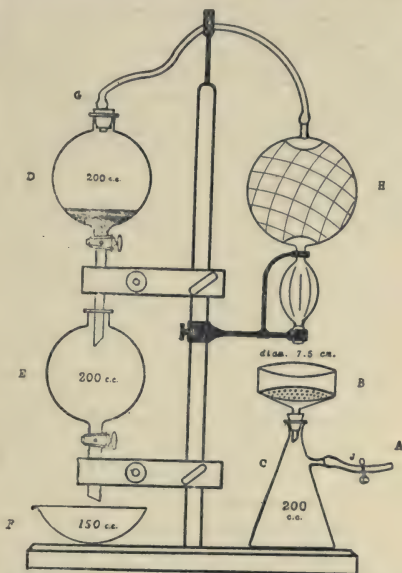


FIG. 53.—Bird's apparatus for the assay of ipecacuanha, belladonna and nuxvomica preparations. H, is an india-rubber bellows; B, is a Buchner's funnel, with a flat filter paper.

PREPARATIONS OF IPECACUANHA.

Acetum Ipecacuanhæ.—Vinegar of ipecacuanha is prepared by mixing 1 part of liquid extract of ipecacuanha, with 2 parts of 90 per cent alcohol and 17 parts of dilute acetic acid. No standards are given, but as the process is merely a mixing of these liquids, no loss should occur, and the arguments as to loss in acetic acid added in the case of Hudson and Bridge (see Vol. II) would hardly apply here. This preparation should contain 0.1 to 0.1125 gm. of alkaloids per 100 c.c. when 50 c.c. (evaporated to 5 c.c.) are assayed by the process described under Extract of Ipecacuanha; 9 per cent of alcohol (absolute) and from 3.6 to 3.7 per cent of acetic acid. Its specific gravity should lie between 0.982 and 0.987.

Liquid Extract of Ipecacuanha.—The official requirement for the liquid extract in the Pharmacopœia is that it should contain from 2 to 2.25 grms. of alkaloids when assayed by the official process, which is described under the root; 20 c.c. of the extract should be used for the determination. It should be pointed out that the Pharmacopœia requires the alkaloids to be weighed and not titrated.

This official process is, however, by no means the best available, and Farr and Wright have strongly recommended the following, which certainly gives very accurate results:—

Five c.c. of the fluid extract is placed on a small porcelain dish, 10 drops of diluted sulphuric acid added, with 5 c.c. of water, and the mixture evaporated over a water bath until the volume of liquid is reduced to about 3 c.c. This is run into a separator, the dish carefully rinsed with 10 drops of water, and then with 15 c.c. of chloroform, the whole being transferred to the separator. An excess of ammonia is added, and the mixture well shaken, and allowed to stand until the chloroform has separated. This is run off, and the agitation and separation repeated with two successive quantities of 5 c.c. of chloroform. The chloroformic solutions are bulked, and the alkaloids extracted by shaking with three successive quantities of 10 c.c. 1 per cent sulphuric acid. The acid alkaloidal solutions are drawn off in turn and mixed. The alkaloids are finally recovered from this solution by repeating the treatment with ammonia and chloroform. The solution of the alkaloids in chloroform is then evaporated in a tared dish over a water bath until all chloroform has been removed. The weight is taken, and the alkaloidal residue titrated with $\frac{\text{HCl}}{10}$ and $\frac{\text{NaHO}}{20}$ as previously described.

The following process, due to Naylor and Bryant, also gives very accurate results:—

Place 10 c.c. of liquid extract in a basin over a warm water bath until the alcohol is dissipated. The solution is transferred to a 50 c.c. flask, and the basin is washed with small portions at a time of a mixture of 2 c.c. of diluted sulphuric acid and 30 c.c. of water. The solution is filtered, and water passed through the filter until the volume measures 50 c.c. Of the filtrate 25 c.c., representing 5 c.c. of liquid extract, are transferred to a separator, together with the

small portions of water used for washing the measure, and the solution is shaken up with 10 c.c. of chloroform. After removal of the separated chloroform the solution is agitated with another 10 c.c. of chloroform which after separation is also withdrawn. The solution is then made alkaline with ammonia, and extracted successively with 3×10 c.c. of chloroform. The chloroform solutions are mixed, evaporated, and the residue weighed and titrated with $\frac{N}{10}$ HCl.

Liquid extract of ipecacuanha has a specific gravity varying between 0.885 to 0.915, and yields from 9 per cent to 12 per cent of extractive matter when dried at 100° . The average alcoholic content is 78 per cent to 79 per cent.

Ipecacuanha Wine.—This is a mixture of one part by volume of the liquid extract with 19 parts of sherry. It should be of the same alkaloidal strength as vinegar of ipecacuanha: 50 or 100 c.c. should be evaporated to 5 c.c. and the assay then carried out as described under the liquid extract. It should contain 20 per cent to 21 per cent of alcohol. This preparation should be tested for salicylic acid, which is sometimes present in sherry. It should be acidified with dilute sulphuric acid and extracted with ether and the ether evaporated. The residue should show no violet coloration with a drop of ferric chloride solution.

Pulvis Ipecacuanhæ Compositus.—Compound ipecacuanha powder or Dover's powder is a mixture of 1 part of ipecacuanha 1 part of opium and 8 parts of potassium sulphate. It should contain 80 per cent to 82 per cent of ash, consisting practically entirely of potassium sulphate. The total alkaloids present when determined as described under opium should be 1.2 per cent.

COLOUR REACTIONS OF THE ISOLATED ALKALOIDS OF IPECACUANHA.

Reagent.	Emetine.	Cephaeline.	Psychotrine.
Ferric chloride.	Indefinite.	Bluish-green. Indefinite.	Pale cherry-red. Indefinite.
Frøehde's reagent.	Dirty green. Bluish.	Pink, changing to green. Reddish-purple.	Pale pink. Dull purple.
Frøehde's reagent and hydrochloric acid.	Grass-green.	Prussian blue.	Pale pink, changing to pale green
Starch and iodic acid.	Negative.	Negative.	Blue.
Ferric chloride and potassium ferricyanide.	Gradual blue coloration.	Almost immediate blue. Immediate blue.	Immediate blue.

Allen and Scott Smith ("Analyst," xxvii. 346) have called attention to the marked resemblance between some of the colour reactions of the alkaloids of ipecacuanha and those of morphine, which might well lead to confusion if qualitative tests were applied for the detection of one or the other alkaloids.

A most valuable means of detecting ipecacuanha alkaloids consists in the production of psychotrine in a crystallized form. Paul and Cownley describe the crystals as well-defined transparent prisms of a pale lemon-yellow colour. Under the microscope, psychotrine forms very minute crystals, which appear to belong to the regular system. Many of them appear to be octahedral, and closely resemble microscopic crystals of arsenious oxide. Other crystals present a remarkable resemblance to granules of rice-starch. Crystals of psychotrine for microscopic observation are readily obtained by shaking out an amyl alcohol or chloroform solution of the alkaloid with a little dilute acetic acid. The acid liquid is separated, concentrated if necessary, and placed in a watch-glass, or, preferably, on a microscopic slide furnished with a cell. A watch-glass or small beaker is then moistened internally with ammonia, and inverted over the alkaloidal acetate solution. After a time the vapours of ammonia are absorbed, and liberate the alkaloid in characteristic crystals, which are observed under the microscope. There is no occasion to employ pure psychotrine for the purpose, the crystals being readily obtainable from the mixed alkaloids of ipecacuanha.

JABORANDI.

The leaves of *Pilocarpus Jaborandi* are official in the Pharmacopœia, but no standards are given.

The principal constituents of this drug are the alkaloids, pilocarpine $C_{11}H_{16}N_2O_2$, isopilocarpine $C_{11}H_{16}N_2O_2$ and pilocarpidine $C_{10}H_{14}N_2O_2$. Pilocarpine is by far the most important of these, and the drug may be regarded as owing its therapeutic activity to this alkaloid, which is described below. It occurs to the extent of about from 0·2 per cent to 0·5 per cent in the leaves.

Jaborandi leaves should yield from 6 per cent to 8 per cent of ash, rarely up to 9·5 per cent.

Tincture of Jaborandi is official. It is prepared by extracting four ounces of the leaves with 45 per cent alcohol, to make 20 fluid ounces of tincture. No standards are given, but a genuine tincture, prepared from leaves of good quality, will have the following characters:—

Specific gravity . . .	0·956 to 0·959
Solid residue . . .	2·6 „ 4·3 grms. per 100 c.c.
Alcohol (by volume) . .	42 „ 43 per cent
Pilocarpine . . .	0·08 „ 0·15 grm. per 100 c.c.

The following process is that of Farr and Wright ("Pharm. Jour." 3, xxii. 1) for the determination of the pilocarpine. Fifty c.c. of the sample to be assayed are introduced into a porcelain dish and evaporated over a water bath, water being added, if necessary, until all spirit is driven off. The alkaloidal liquor is allowed to cool, 1 c.c. of semi-normal sulphuric acid added, and the solution filtered through cotton wool, the dish being rinsed with acidulated water, and the rinsings added to the filtered liquid. The latter is then rendered alkaline by the addition of 2 c.c. of B.P. liquor ammoniæ,

and the liberated alkaloid taken out by agitation with two successive quantities of 15 c.c. of chloroform.

To obtain the alkaloid in a pure condition, it is withdrawn from solution in chloroform by shaking with acidulated water, 25 c.c. of distilled water being acidified with 2 c.c. of semi-normal sulphuric acid, and added in three successive portions. The mixed acid solutions are again rendered alkaline with ammonia, and shaken with two successive quantities of 15 c.c. of chloroform. The chloroformic alkaloidal solution is then agitated with a little slightly ammoniated water, and after separation is drawn off and evaporated, and the residue heated in a water oven at 100° till the weight is constant.

The amount of alkaloid may be checked by dissolving the residue in a calculated excess of one-twentieth normal hydrochloric acid, and titrating the excess of acid with one-twentieth normal soda or baryta solution, using iodeosine or methyl-orange as indicator. Each c.c. of one-twentieth normal acid is equivalent to 0.0104 grm. of pilocarpine.

The amount of mucilaginous matter present in the tincture is so great as to produce emulsification of the chloroform when that liquid is shaken up with it, and it is therefore necessary to remove such matters by adding strong alcohol, before proceeding with the estimation of the tincture.

Fluid extract of Jaborandi is five times the strength of the tincture and should have the following characters (which are not official):—

Specific gravity . . .	1.020 to 1.050
Solid residue . . .	21 „ 22 grms. per 100 c.c.
Alcohol by volume . . .	33 „ 35 per cent
Pilocarpine . . .	0.2 „ 0.75 grm. per 100 c.c.

Pilocarpine $C_{11}H_{16}N_2O_2$ has not been obtained in the crystalline condition, but only as a thick syrup. It is official in the form of its nitrate, which, as in the case with the hydrochloride, forms well-defined crystals. The Pharmacopœia describes pilocarpine nitrate $C_{11}H_{16}N_2O_2 \cdot HNO_3$ as a white crystalline powder, soluble in 8 parts to 9 parts of cold water and freely soluble in hot alcohol. Strong sulphuric acid forms with it a yellowish solution, which on the addition of potassium bichromate gradually acquires an emerald-green colour. It leaves no ash when burned.

Pilocarpine hydrochloride $C_{11}H_{16}N_2O_2 \cdot HCl$ is not official, but is a salt sometimes met with in pharmacy.

Pilocarpine nitrate, when rendered alkaline, and the free alkaloid extracted with chloroform, should yield 76.75 per cent of free pilocarpine. The nitrate should melt at 173° to 175° and in aqueous solution should show a specific rotatory power of about + 88°; a concentrated aqueous solution does not yield a precipitate with ammonia or caustic soda solution. Ten or 20 milligrams dissolved in 2 c.c. of water and 2 c.c. of slightly acidified hydrogen peroxide added, and 5 c.c. of benzol added, and finally 3 or 4 drops of a dilute solution of potassium bichromate (1 in 300), and the mixture gently shaken, the benzol layer will acquire a violet colour and the aqueous layer will be yellow. If the pure alkaloid be separated as above mentioned,

it should have a specific rotation in aqueous solution of $+101^\circ$. It possesses both acid and basic properties, and forms a crystalline picrate melting at 147° .

LOBELIA.

The dried flowering herb of *Lobelia inflata* is official, but no standards are given.

The principal constituent is the liquid alkaloid lobeline $C_{18}H_{23}NO$ (?) which can be determined with comparative accuracy.

The drug yields from 10 per cent to 12 per cent of ash.

The alkaloid may be determined in the same manner as coniine in hemlock fruits (p. 544). One c.c. of $\frac{N}{20}$ HCl is equivalent to 0.01425 grm. of alkaloid, calculated as lobeline.

Ethereal tincture of lobelia is an official preparation made by exhausting 4 ounces of the drug by a mixture of 1 volume of ether and 2 of 90 per cent alcohol, the resulting product measuring 1 pint. There are no standards given. A genuine tincture should have the following characters:—

Specific gravity	0.812 to 0.817
Solid residue	0.9 „ 1.5 grms. per 100 c.c.
Alkaloids	0.02 „ 0.04 per cent

By careful fractionation 30 per cent should be obtained boiling below 50° , indicating the presence of a due proportion of ether.

The alkaloids are determined in the same manner as in the case of tincture of conium.

JALAP.

Jalap consists of the dried root tubercles of *Ipomœa purga*. It is official in the British Pharmacopœia, and is required by that authority to contain from 9 to 11 per cent of resin, when assayed by the following process:—

Ten grams of the jalap in powder are digested with 20 c.c. of 90 per cent alcohol in a covered vessel, heated gently for twenty-four hours. It is then transferred to a small percolating apparatus and exhausted with alcohol. Five c.c. of water are added to the alcoholic extract, and the alcohol removed by distillation. The residue, whilst still hot, is transferred to an open dish, allowed to cool, and the separated resin washed several times with water, dried, and weighed.

This resin must not yield more than 10 per cent to ether, indicating absence of scammony and Tampico jalap resins; and an alcoholic solution should not yield a blue-green colour with solution of ferric chloride, indicating the absence of guaiacum resin.

The principal constituent of jalap is generally said to be the so-called jalap resin or jalapin, which is essentially a glucoside of the formula $C_{54}H_{96}O_{27}$.

This body, also known as jalapurgin or convolvulin, must not be confused with the glucoside of *Ipomœa simulans*, the Tampico jalap,

which is often termed jalapin, but is probably identical with scammonin. When pure, jalapin (*Jalapurgin, convolvulin*) is a white amorphous powder, almost if not quite insoluble in ether, petroleum ether, benzene or water; slightly soluble in chloroform, and easily so in alcohol and acetic acid. It melts between 150° and 155° . It reduces ammoniacal silver nitrate solution on warming, and after boiling with dilute acids, the reaction products reduce Fehling's solution. The products of hydrolysis are glucose, methyl-ethyl acetic acid, purgic acid $C_{25}H_{46}O_{12}$ and convolvulic acid $C_{45}H_{80}O_{28}$.

Jalapurgin dissolves in sulphuric acid with a fine red coloration.

Recent researches by Power and Rogerson ("Pharm. Jour." 1909 [iv.] 29, 7) indicate that jalap resin is of a much more complex composition, and that its physiologically active components are all indefinite and amorphous, and that there is no justification for the formulæ usually assigned to them. From jalap resin, a small quantity of ipurganol $C_{21}H_{32}O_2(OH)_2$ was isolated, as well as β -methyl-aesculetin.

The Examination of Jalap.—The examination of the whole tubers is practically confined to an estimation of the amount of resin present, and an examination of the resin itself. In the case of powdered jalap, this should be supplemented by the estimation of the ash and a microscopic examination.

The ash of jalap should not exceed 6.5 per cent.

The Resin Value of Jalap.—A large number of samples do not contain as much resin as required by the British Pharmacopœia. Such samples, however, can be used for the manufacture of "jalapin," and as the tincture of jalap of the Pharmacopœia is a standardized preparation, it does not appear to be of much importance whether a weaker jalap is used in its preparation. It would certainly be advisable to reduce the official standard to a minimum of about 7.5 per cent.

Alcock ("Pharm. Jour." 3, xxii. 107) prefers the following process for the determination of resin. In the author's experience it is a better process than that of the Pharmacopœia, in that less extraneous matter is extracted by amyl alcohol than by ethyl alcohol, and it obviates loss of resin which may become attached in films to the dish in which it is washed with hot water. This process is as follows:—

Place 1 grm. of powdered jalap—free from agglutinated lumps—in a suitable bottle, add 20 c.c. of amylic alcohol, and shake well from time to time. After a few hours, strain the liquid off through a little cotton wool into a glass separator, wash out the bottle with 5 c.c. of amylic alcohol, and place the washings on the marc in the funnel; repeat with 5 c.c. more if necessary, so as to ensure the presence of all the resin in the separator.

Now shake up the amylic solution of the resin with small quantities of water at 50° C., set aside for the liquids to separate, remove the lower aqueous layer, and repeat the washing with water until nothing more of a non-resinous nature is removed. Afterwards transfer the solution of the resin to a weighed dish containing 10 c.c. of distilled water, wash out the separator with a little amylic alcohol, placing the washings in the dish, evaporate on a water bath in the usual way, and when dry, weigh.

After the resin is extracted it should be powdered and a weighed quantity exhausted with anhydrous ether. If more than about 10 per cent be dissolved, admixture with foreign bodies, such as Tampico-jalap, is to be suspected.

Four samples of genuine jalap were extracted by the author and the resins examined. They were found to have the following characters, after drying at 105° :—

Soluble in ether . . .	4.95 per cent	6.22 per cent	5.12 per cent	5.3 per cent
Soluble in alcohol . .	Complete	Complete	Complete	Complete
Acid value	14.6	13.0	15.0	16.5
Ester value	116	124	120	122

These results are in agreement with those of Kremel and Beckurts. Commercial "jalapin" should have substantially the above characters.

Microscopic Characters.—Powdered jalap should be examined both in its natural condition, and after being bleached with sodium hypo-



FIG. 54.—Powdered jalap.

chlorite. Many rounded cells containing starch grains will be found, and many dark, somewhat angular, resin cells. Parenchymatous

cells, fibres and sclerotic cells with very thick walls are to be observed. The starch granules are circular and flattened, or oyster-shaped. The hilum is distinct and a few concentric rings can be traced. Anything more than quite a small proportion of pitted vessels and wood fibres should be regarded with suspicion.

Tincture of Jalap.—The official tincture of jalap is an extract of jalap with 70 per cent alcohol, of such strength that when assayed by the process for determining the resin in jalap, it contains from 0.145 to 0.155 gram of resin in 10 c.c.

The specific gravity of a properly prepared tincture lies between 0.910 to 0.915. The solid residue should not be less than 3.5 per cent nor more than 4.7 grms. per 100 c.c.; and the alcoholic strength should not be less than 65 to 66 per cent.

NUX VOMICA.

This drug, which is official in the Pharmacopœia, consists of the dried ripe seeds of *Strychnos nux vomica*.

There are no standards in that authority for the drug, its preparations being standardized to a given amount of strychnine.

As the seeds, which are from three-quarters of an inch to one inch in diameter, are almost entirely sold whole, the analyst has rarely to consider the question of adulteration, except in so far as from time to time a false nux vomica is to be found mixed with the true seeds.

The examination of the seeds is therefore usually confined to the determination of the strychnine present, or sometimes the brucine also.

Strychnine and brucine are the two characteristic alkaloids of this drug, the strychnine being the more important.

Nux vomica should yield from 2 per cent to 2.5 per cent of ash on incineration.

Strychnine $C_{21}H_{22}N_2O_2$ is dealt with on page 578. It is present in the seeds to the extent of 0.7 per cent to 1.60 per cent.

Brucine $C_{23}H_{26}N_2O_4$, is possibly a dimethyl-strychnine, and is a bitter, white, odourless, crystalline compound, usually containing four molecules of water. It melts at about 115°. It is less poisonous than strychnine. The most satisfactory reaction for this alkaloid is the following. On adding a drop or two of cold concentrated nitric acid to an ether-chloroform residue or any other solid matter containing brucine, a scarlet or blood-red colour is produced which on heating changes to yellow. If the mixture be then cooled and heated with a trace of stannous chloride or sodium thiosulphate, a purple colour results, which is destroyed by excess of either HNO_3 or stannous chloride. From the analyst's point of view, brucine is only important in reference to its separation from strychnine.

Assay of Nux Vomica.—The process described as official under liquid extract of nux vomica is the result of work by Dunstan and Short ("Pharm. Jour." 3, xiv. 290). It has, however, been shown that brucine ferrocyanide is not completely soluble in acidulated water,

and that the ferrocyanide salts are very unstable. It is also probable that some of the strychnine is carried into solution in the course of the washing. Farr and Wright have shown by an exhaustive series of experiments that the official process (see page 578) is sufficiently accurate for all practical purposes, but that not more than 5 c.c. of the liquid extract should be used, and not more than 30 c.c. of the tincture. They improve the process, however, by using 200 c.c. of wash water at 100° F., and making an allowance for the strychnine dissolved. This may be taken as 0.002 gm. per 100 c.c.

Beckurts ("Pharm. Post," 18, 67, and "Apoth. Zeit." 1891, 537) prefers the following method of assay:—

Ten grms. of powdered seeds are exhausted with about 45 per cent alcohol and the percolate evaporated to a thin syrup. This is dissolved in a mixture of 10 c.c. of alcohol, 5 c.c. of water and 5 c.c. of ammonia (10 per cent). The alkaloids are shaken out with chloroform. The alkaloids are then weighed, or titrated, but no separation of the two bases is attempted.

F. C. J. Bird has found the following to give most accurate results:—

Nux vomica in powder	5 grms.
Solution of potash, 10 per cent	2 c.c.

Triturate in a mortar until uniformly moistened

Amyl alcohol, 1 vol.	} a sufficient quantity.
(Solvent) Chloroform, 3 vo's.	
Ether, 4 vols.	

Add the moistened powder to 20 c.c. of the above solvent, previously placed in a separator plugged with cotton wool, and macerate for half an hour with occasional agitation. Adapt a pressure-ball to the separator and force out the liquid as completely as possible by air pressure. Add sufficient solvent to just cover the powder, insert the stopper of the separator, agitate vigorously, let stand fifteen minutes and again force out the liquid. Repeat this until no more alkaloid is extracted, as shown by evaporating a few drops and testing with diluted acid and Mayer's reagent. Usually five to six extractions will be found sufficient.

Agitate the mixed ethereal extracts with:—

Diluted sulphuric acid, 6 c.c.	} 11, 10, and 10 c.c.
Water, 25 c.c.	

in three successive quantities. Transfer the united acid liquids to a 200 c.c. separator half filled with water at 70° F. (21.1° C.), and having the neck above the stopcock plugged with a very small pledget of cotton wool. Add a freshly prepared solution of

Potassium ferrocyanide	1.25 grms.
Water	25 c.c.

and completely fill the separator with water at 70° F. (21° C.) Replace the stopper by a cork carrying a thermometer; if necessary raise the temperature of the contents to 70° F., by rotating the separator in the steam of a water bath. Agitate occasionally during half

an hour, then allow to remain at rest for an additional hour and a half, maintaining the temperature of the liquid at 70° F. by occasional warming when necessary. (At 70° F. precipitation of strychnine ferrocyanide invariably commences well within a minute after the addition of the potassium ferrocyanide solution.) Adapt an air-pressure ball to the separator and force out the mother liquor.

Diluted sulphuric acid	5 c.c.	} At 100° F. (37·7° C.)
Water	195 c.c.	

Add about 50 c.c. of the above wash water to the precipitate, rotate, and apply air pressure as before, regulating the flow of liquid by the stopcock to a quick succession of drops. Then add the remainder of the wash water, agitate and repeat. Insert the stopper of the separator, invert and displace the cotton wool plug by means of a stiff wire passed through the open stopcock. Then add

Water	10 c.c.
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Agitate to diffuse the precipitate, and add

Chloroform	7·5 c.c.
Strong solution of ammonia	2 c.c.

Shake well and separate. Repeat with

Chloroform	7·5 c.c.
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and again separate. To the mixed chloroformic solutions in a tared glass dish (preferably with a flat bottom) add

Amylic alcohol	2 c.c.
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(This prevents decrepitation.)

Evaporate on a water bath and dry the residue to a constant weight. Add 8 mg. to the weight of the strychnine thus obtained (to compensate for strychnine ferrocyanide lost in the wash water) and multiply the result by 20.

Extract of Nux Vomica.—Two grms. of the triturated extract are agitated with 5 c.c. of ammonia, 5 c.c. of water, and 10 c.c. of alcohol until solution is effected; the solution is then shaken with three portions of chloroform, 20 c.c., 10 c.c., and 10 c.c. The united chloroform solutions are evaporated or the chloroform distilled off, the residue warmed upon a water bath for several minutes with $\frac{N}{10}$ hydrochloric acid, then filtered, and the filter thoroughly washed.

The filtrate is titrated with $\frac{N}{100}$ alkali, using cochineal as the indicator; if the number of c.c. of alkali be subtracted from 150 (corresponding to 15 c.c. of $\frac{N}{10}$ acid), and the remainder multiplied by 0·00364 (assuming that the alkaloids are present in equal amounts), the product will represent the total alkaloid present in 2 grms. of extract; multiplying this by fifty will give the percentage.

SEPARATION OF STRYCHNINE FROM BRUCINE.

Lyons ("Pharm. Review," 20, 253) separates the brucine from the strychnine—in the total alkaloids obtained, for example, by the above process, in the following manner.

Advantage is taken of the fact that while strychnine sulphate is practically insoluble in 10 per cent H_2SO_4 , brucine sulphate is very soluble. Working with an experimental mixture of strychnine, 45, and brucine, 55, portions varying in amount from 50 to 150 mg. of total alkaloid were taken and treated with 10 per cent H_2SO_4 in the proportion of 1 c.c. to every 10 mg. of alkaloids. After constant agitation for ten minutes, and then at frequent intervals for two hours, the solution is passed through a small filter, washed with a few drops of H_2SO_4 10 per cent; and the strychnine sulphate left on the filter, decomposed with ammonia and extracted with chloroform. In each case the loss of strychnine was found to be about 1.75 mg. for each c.c. of acid used.

To determine the proportion of strychnine in the total alkaloid extracted from *nux vomica* or its preparations, the above process is thus conducted: For each 15 mg. of alkaloids 1 c.c. of 10 per cent H_2SO_4 is added in a capsule and frequently agitated for at least one hour. The mixture is then filtered, the insoluble residue being entirely transferred to the filter, and washed with 1 c.c. of acid. The filter and its contents are then replaced in the capsule and treated with 10 c.c. of CHCl_3 and 3 c.c. of 10 per cent ammonia, agitated with a glass stirrer until all the alkaloid is dissolved and the liquid transferred to a separator. The filter is then washed with two successive washings, each of 5 c.c. of CHCl_3 , which are added to the rest in the separator. The chloroform solution is then received in a tared capsule, evaporated to dryness, after adding 2 c.c. of alcohol, dried to constant weight and weighed. To the weight obtained 1.75 mg. is added for each c.c. of acid used, the result being the strychnine present in the total alkaloids.

The method adopted in the United States Pharmacopœia depends upon the destruction of the brucine by means of nitric acid. The total alkaloids are extracted in the usual manner from 10 c.c. of fluid extract, and dissolved in 15 c.c. of 3 per cent sulphuric acid and cooled. To this is added 3 c.c. of a mixture of equal parts of nitric acid and distilled water, and the whole set aside for ten minutes. (Farr and Wright suggest that the mixture be heated to 50°C ., which ensures the complete destruction of the brucine.) The mixture is then placed in a separator, 25 c.c. of 10 per cent solution of soda added, and the strychnine extracted with three quantities of chloroform (20 c.c., 10 c.c. and 10 c.c.). The chloroform solutions are evaporated to dryness, the residue is dissolved in 10 c.c. of decinormal sulphuric acid and the solution titrated with fiftieth normal sodium hydrate. Each c.c. of decinormal sulphuric acid absorbed corresponds to 0.0332 grm. of strychnine. It is preferable to use twentieth normal solutions in each case.

This method gives results corresponding very closely with the method official in the British Pharmacopœia.

Liquid Extract of Nux Vomica.—This is the principal official pre-

paration of *nux vomica*, and is a diluted alcoholic extract of the drug which should contain 1·5 grms. of strychnine per 100 c.c. when assayed by the official process.

A properly prepared extract should have a specific gravity of 0·945 to 0·965 and should contain 11 to 12·5 grms. of solid matter per 100 c.c. It should contain 61 to 63 per cent of alcohol (by volume).

The following is the official process of assay, except that half the quantities are given, as being more accurate in the result. Evaporate 5 c.c. to a thick syrup on the water bath, dissolve the residue in 10 c.c. of water with gentle heat. Place the solution in a separator and add 2·5 grms. of sodium carbonate dissolved in 12·5 c.c. of water, and 5 c.c. of chloroform, and then agitate well. Run off the chloroform when separated, and repeat the extraction with chloroform twice. Extract the mixed chloroform solutions with 5 c.c. of about 3 per cent sulphuric acid three times, and dilute the united acid liquids to 88 c.c. Transfer to a stoppered flask, and add 12·5 c.c. of a 5 per cent solution of potassium ferrocyanide. Shake well and frequently for a minute or so at intervals during half an hour. Allow to stand for six hours, and then collect the precipitate on a small filter, rinsing out the last portions from the flask with water containing one-fifth per cent of sulphuric acid, and wash until the washings are free from bitterness. Wash the precipitate into a separator, and add 2·5 c.c. of solution of ammonia (10 per cent). After well shaking add 5 c.c. of chloroform and shake well then add a further 2·5 c.c. and after well shaking separate the chloroform and allow the chloroform to evaporate in a current of warm air in a tared dish, and then dry on the water bath for an hour, taking care that the dish is covered as otherwise loss will take place owing to decrepitation. The resulting strychnine is then weighed.

Extract of Nux Vomica is a semi-solid preparation made by evaporating the liquid extract with sugar of milk so that the resulting extract should contain 5 per cent of strychnine. It is assayed in the same manner as the liquid extract, except that there is no preliminary evaporation of alcohol, or in the manner described on p. 576.

Tincture of Nux Vomica is prepared by diluting 2 fluid parts of the liquid extract with 3 of distilled water, and 7 of 90 per cent alcohol.

It should contain, when assayed by the official process above described, from 0·24 gm. to 0·26 gm. of strychnine per 100 c.c.

It has a specific gravity about 0·910 to 0·915 and should contain 60 per cent of alcohol by volume, and 1·7 grms. of solid matter per 100 c.c.

Strychnine.— $C_{21}H_{22}N_2O_2$ is one of the alkaloids official in the British Pharmacopœia. The official requirements for it are sufficient to ensure it being of comparative purity. It is described as occurring in trimetric prisms, soluble in 150 parts of cold water and in 6 parts of chloroform and in 40 parts of boiling absolute alcohol. It is nearly insoluble in ether. Sulphuric acid forms with it a colourless solution, which, on the addition of a crystal of potassium bichromate, acquires an intensely violet hue, speedily passing through red to yellow. With sulphuric acid containing $\frac{1}{2000}$ part of potassium permanganate, a minute particle of strychnine gives a violet coloration. It is not coloured by nitric acid (absence of brucine) and leaves no ash.

Strychnine melts at 265° to 266°.

The only salt that is official in the Pharmacopœia is the hydrochloride $C_{21}H_{22}N_2O_3 \cdot HCl, 2H_2O$. If dried at 100° C., it should contain from 7.3 per cent to 8.8 per cent of water.

The detection of strychnine. The following qualitative tests are suitable for the detection of strychnine, which is best extracted from substances supposed to contain it by a mixture of equal parts of chloroform and ether, after the addition of ammonia:—

(1) A solution of sodium phosphomolybdate in nitric acid precipitates strychnine as a yellowish amorphous mass from complex organic liquids. The precipitate (which may consist of other alkaloids) is separated, and treated with dilute ammonia, and the liquid extracted with ether-chloroform and tested by the colour reactions given below.

(2) A solution of strychnine as dilute as 1 in 105,000 gives a red-brown precipitate with iodine in potassium iodide solution.

Mayer's reagent gives a precipitate in solutions as dilute as 1 in 150,000.

(3) Potassium ferrocyanide precipitates strychnine as a white or faintly yellow crystalline powder.

(4) Strychnine in a very minute quantity, moistened with sulphuric acid, gives a deep violet colour, (due to an oxidation reaction) when a small crystal of potassium bichromate is brought into contact with it. The colour is transient and is rapidly changed and masked by the green of the chromium salt. A drop of *very* dilute solution of permanganate of potassium gives the reaction with more distinctness. Lead and manganese dioxide are equally effective. Potassium ferricyanide also gives the reaction, but probably the best reaction is obtained with a 1 per cent solution of ammonium vanadate in sulphuric acid, with which the strychnine is moistened. Cerosceric oxide Ce_3O_4 is also very effective. According to Allen the reaction is best obtained as follows:—

The solution of the strychnine in ether-chloroform should be evaporated in a porcelain dish. If the quantity of strychnine is likely to be very small the dish should be immersed in hot water and the ether-chloroform solution dropped slowly into the dish from a burette so as to allow the solvent to evaporate rapidly so as to concentrate the residue on a small spot. The cold residue should be treated with 2 or 3 drops of pure concentrated sulphuric acid, which should be mixed with it with a glass rod. The mixture should be allowed to stand for five minutes in order to note whether any coloration be produced. If any marked colour is produced the dish should be gently heated (not to boiling-point of water) for half an hour, the contents diluted with water, filtered, made alkaline with ammonia, and the strychnine again recovered by ether-chloroform, and the solvent evaporated in the same manner. This residue is again treated with a drop or two of sulphuric acid. The oxidizing agent (which Allen prefers to be manganese or lead dioxide) is then moistened with the sulphuric acid solution by means of a glass rod, and the mixture stirred. The blue-violet colour will be at once developed, passing to purple and then to

cherry red, the last tint being fairly persistent. This reaction will detect $\frac{1}{200000}$ of a grain of strychnine.

(5) If a trace of solid strychnine be dissolved in dilute nitric acid, the liquid gently heated, and a minute particle of potassium chlorate then added, an intense scarlet colour results.

R. H. Davies has proposed the following method for the estimation of small quantities of strychnine. The results, however, are only approximate.

He modifies the chromate test in such a way as to make it applicable also for approximate quantitative estimations of traces of this alkaloid. A very weak solution of potassium bichromate in strong sulphuric acid is placed in a test-tube, and the solution of the strychnine then added to it, when the reaction can be readily observed. The colour thus produced soon disappears, giving place to a reddish-orange, which is fairly persistent. This coloration is compared with those obtained under the same conditions with exceedingly weak strychnine solutions of various but known strengths. An approximate idea of the amount of this alkaloid in the solution under examination is then arrived at by a colorimetric process similar to Nesslerizing.

Liquor Strychninæ hydrochloridi.—The Pharmacopœia recognizes a solution of the hydrochloride of strychnine, which contains 17·5 grains of the salt in 4 fluid ounces of alcohol (22·5 per cent by volume). It should have a specific gravity of about 0·970, and, after evaporating most of the alcohol, on the addition of ammonia and extraction with ether-chloroform, should yield strychnine equivalent to 1 grm. of the hydrochloride per 100 c.c.

Easton's Syrup and Syrup of Hypophosphites.—Under the name of Easton's syrup, a preparation containing phosphates of iron, quinine and strychnine is largely sold.

The Pharmacopœia has not recognized the name "Easton," but contains a preparation of the same nature, under the name "Syrup of Phosphate of Iron, with Quinine and Strychnine". This syrup should contain 1 grain of dry ferrous phosphate, 0·8 grain of quinine calculated as sulphate and $\frac{1}{32}$ of a grain of strychnine, per fluid drachm.

The syrup known as "compound syrup of hypophosphites" is a similar preparation, but contains hypophosphorous acid instead of phosphoric acid. It is not official in the Pharmacopœia.

In examining these preparations the iron is determined in the usual way, on the dried and ignited residue of 5 grains of the syrup. Free phosphoric acid is determined by titration with caustic soda using methyl-orange as an indicator.

The alkaloids are best determined by the following process, due in the main to Harrison and Gair.

The alkaloids are extracted from 150 c.c. of the syrup by diluting with 250 c.c. of water, adding a little citric acid and ammonia and extracting with ether-chloroform. The alkaloids obtained on evaporating the solvent are dissolved in 60 c.c. of water slightly acidulated with sulphuric acid; ammonia is added as long as the precipitate redissolves. Fifteen grms. of powdered sodium potassium tartrate are then added gradually with stirring; then more ammonia, until the

mixture is only just acid to litmus paper, and it is then warmed on the water bath for about fifteen minutes, and allowed to stand till quite cold (about two hours). The quinine tartrate is then filtered off with the aid of a pump, and washed with a solution of 15 grms. sodium potassium tartrate in 45 c.c. of water, made just acid with sulphuric acid. The filtrate and washings are mixed, made strongly alkaline with ammonia, and extracted three or four times with chloroform; the chloroformic solution is washed with 10 c.c. of water, containing a few drops of ammonia solution, evaporated to about 4 or 5 c.c., 10 c.c. of alcohol added, and the mixture evaporated to dryness; the residual alkaloid is washed three times with 1 c.c. each time of washed ether, and the washings rejected; the residue is practically pure strychnine, and is dried and weighed. The alcohol is added not only to prevent decrepitation, but also to avoid retention of chloroform by the strychnine, which otherwise occurs. If the amount of strychnine in the total alkaloid taken is much over 0.1 gm. it is necessary to increase the quantity of the first solution and of the Rochelle salt, otherwise the same treatment is employed.

OPIUM AND ITS PREPARATIONS.

Opium is the inspissated juice of the unripe fruit capsules of *Papaver somniferum*. It is produced in various countries, notably Persia, India, China, Asia Minor and Turkey.

Practically the whole of the supply of opium consumed in this country is derived from Turkey and Asia Minor on the one hand, and Persia on the other.

So-called Turkey opium is the variety principally employed for preparations in pharmacy and is the usual "druggist's opium". It occurs in rounded or flattened cakes covered with the small triangular fruits of a species of rumex. Persian opium is the variety principally used by manufacturers of morphia and other opium alkaloids. It is imported in conical or more or less brick-shaped masses wrapped in red paper, and, rarely, in sticks or flat cakes wrapped in white paper. The characteristic difference in the appearance of these two varieties is that Turkey opium is granular, whereas Persian opium is a homogeneous mass.

The Composition of Opium.—The active constituents of opium consist of a series of alkaloids, the remainder consisting of water, insoluble inert matter and extractives and colouring matter. The well-defined alkaloids of opium are as follows: morphine, narcotine, codeine, thebaine, narceine, papaverine, meconidine, codamine, laudanine, laudanoline, lanthopine, protopine and several others. The only ones which are employed to any great extent in medicine are morphine and codeine, which will be discussed later. The indifferent matter of opium consists of mucilage, sugar, wax, resins and mineral matter.

The Adulteration of Opium.—The adulteration of opium may be divided into two types. In the cakes or lumps, there may be found from time to time, coarse adulterants such as shot, stones, gravel,

pieces of metal and the like; but the principal adulterants are organic matters of various types which are beaten up with the juice before it is dried. Writing of Indian opium fifty years ago Dr. Eatwell said: "Flour is a very favourite article of adulteration, but is readily detected; opium so adulterated speedily becomes sour, it breaks with a peculiar, short, ragged fracture, the sharp edges of which are dull and not pink and translucent as they should be; and on squeezing a mass of the drug after immersion in water, the starch may be seen oozing from the surface." The application of the iodine test, however, furnishes conclusive evidence of the presence of an amylaceous compound. The farina of the boiled potato is not unfrequently made use of; impure treacle is also occasionally used. In addition to the above, a variety of vegetable juices, extracts, pulps and colouring matters, are occasionally fraudulently mixed with the opium; such are the inspissated juice of the common prickly pear, and the extracts produced from various other narcotic plants. The juicy exudations from various plants are frequently used, and of pulps, the most frequently employed are those of the tamarind and of the bael fruit.

There is no doubt that opium is still considerably adulterated, but as its morphia content forms the basis of its value, and practically all the opium used in this country is sold and used on that basis, admixture with inert foreign matter is not a very serious matter.

Genuine Turkey opium contains from 8 to 13 per cent—rarely up to 16 per cent of morphine, calculated on the dry drug. Persian opium contains in the natural state from 7·5 to 13 per cent of morphine, rarely up to 16 per cent. It is probable that absolutely pure Persian opium contains from 10 to 15 per cent of morphine as its usual quantity. Codeine exists in the proportion of 0·3 to 2 per cent, Persian opium containing considerably more than Turkey opium. Narcotine exists to the extent of 3 to 8 or 9 per cent, the remaining alkaloids only forming about 1 per cent of the opium.

The Analysis of Opium.—The analysis of opium is, in practice, confined almost entirely to the determination of morphine. At times a fuller analysis is required, however. The figures on opposite page were obtained in six samples each of typical Turkey and Persian opiums.

The insoluble residue of pure opium, when examined under the microscope, should only contain quite small quantities of starch and only a small amount of the cellular matter of the outer epidermis of the poppy capsule. Persian opium usually contains more starch than does Turkey opium.

The Estimation of Morphine.—Many processes have been published for the determination of morphine, but it is proposed to here give details of a few which after exhaustive examination have proved to give good results. The official process of the British Pharmacopœia, however, must be described, for, although open to much criticism, it is the official process which the manufacturing druggist is bound to employ. This process is as follows:—

Take the following quantities:—

Opium dried at 212° F. (100° C.)	}	14	grms.
and in No. 50 powder				
Calcium hydroxide, freshly prepared	.	.	.	6 "
Ammonium chloride	.	.	.	4 "
Alcohol (90 per cent)	}		of each a sufficient quantity.
Ether				
Distilled water				

Triturate together the opium, calcium hydroxide, and 40 c.c. of water in a mortar until a uniform mixture results; add 100 c.c. of water and stir occasionally during half an hour. Filter the mixture through a plaited filter, about 10 cm. in diameter, into a wide-mouthed bottle having a capacity of about 300 c.c., and marked at exactly 104 c.c., until the filtrate reaches this mark. To the filtered liquid (representing 10 grms. of opium) add 10 c.c. of alcohol (90 per cent) and 50 c.c. of ether; shake the mixture; add the ammonium chloride, shake well and frequently during half an hour; set aside for twelve hours for the morphine to separate. Counterbalance two small filters; place one within the other in a small funnel in such a way that the triple fold of the inner filter shall be superposed upon the single fold

TURKEY OPIUM.

Moisture.	Mineral Matter.	Aqueous Extract.	Matter Insoluble in H ₂ O.	Anhydrous Morphine.
Per cent	Per cent	Per cent	Per cent	Per cent
16·4	5·1	49·9	33·7	11·15
20·2	3·9	50·2	29·6	8·56
18·0	3·6	53·6	28·4	9·20
16·9	4·4	46·2	36·9	12·31
17·8	4·8	49·9	32·3	10·97
21·5	4·3	55·0	23·5	9·95
PERSIAN OPIUM.				
10·5	4·8	59·4	30·1	11·25
14·8	3·2	57·9	27·3	9·40
28·2	3·1	55·0	16·8	8·0
17·6	4·6	57·8	24·6	10·35
18·1	4·8	55·9	26·0	10·80
14·5	5·1	60·0	25·5	11·65

of the outer filter; wet them with ether; remove the ethereal layer of the liquid in the bottle as completely as possible by means of a small pipette, transferring the liquid to the filter; rinse the bottle with 20 c.c. of ether, again transferring the ethereal layer, by means of the pipette, to the filter; wash the filter with a total of 10 c.c. of ether, added slowly and in portions. Let the filter dry in the air, and pour upon it the contents of the bottle in portions, in such a way as to transfer the granular crystalline morphine from the bottle with morphinated water, until the whole has been removed. Wash the crystals with morphinated water until the washings are free from colour; allow the filter to drain, and dry it, first by pressing between

sheets of bibulous paper, afterwards at a temperature between 131° and 140° F. (55° C. and 60° C.) finally at 230° F. (110° C.) for two hours. Weigh the crystals in the inner filter, counterbalancing by the outer filter. Take 0.5 gm. of the crystals and titrate with decinormal volumetric solution of sulphuric acid until the liquid, after boiling, slightly reddens blue litmus paper. One c.c. of this volumetric solution represents 0.0285 gm. of pure anhydrous morphine. The weight of pure anhydrous morphine indicated by the titration + 0.104 gm. (representing the average loss of morphine during the process), should amount in total to 1 gm., that is to say, to a total of not less than 0.95 gm. and not more than 1.05 grms., corresponding to about 10 per cent of anhydrous morphine in the dry powdered opium.

Opium is one of the standardized preparations of the British Pharmacopœia (see Vol. II, under "standardization"). It directs that any suitable variety of opium may be used for the preparation of the tincture and extract of opium, provided that when dry it contains at least 7.5 per cent of anhydrous morphine. But when otherwise used for officially recognized purposes, opium is to be of such a strength that when dried and powdered it contains from 9.5 per cent to 10.5 per cent of morphine. Opium stronger than this is to be diluted either with a weaker opium of from 7.5 per cent to 10 per cent strength, or with sugar of milk.

The following process is probably the most accurate for the determination of morphine. It is based on the work of Prollius and Flückiger, with modifications by various other chemists. Ten grms. of the opium are exhausted by digestion for half an hour, after disintegration, with 100 c.c. of water at about 40° C. After the disintegrated matter has settled, the liquid is decanted as completely as possible, and then the solid matter poured on to the filter. After this has drained well, the solid matter is washed back into the beaker in which it was digested, and the process repeated. The solid matter on the filter is now washed with warm water to complete exhaustion, and the mixed filtrates evaporated to a thin syrup on the water bath. The liquid is transferred to a flask, the dish being rinsed out with warm water, and 2.5 c.c. of 90 per cent alcohol are added, and 30 c.c. of ether. The flask is corked, and the contents are gently shaken to ensure as complete mixture as possible, when 2.5 c.c. of ammonia (specific gravity 0.935) are added. The flask is well shaken so as to cause the morphia to be precipitated in the most convenient form, and allowed to stand for eighteen hours with occasional shaking. The liquid is then filtered, preferably with the aid of a pump, and the precipitate is dried and washed with benzene to remove narcotine and other alkaloids. It is then dried and weighed, but will contain up to 10 per cent of impurities, so that the weight is only taken as a check. It is then titrated with decinormal sulphuric acid using litmus as an indicator. One c.c. of decinormal sulphuric acid = 0.0303 gm. of hydrated morphine or 0.0285 of anhydrous morphine.

Dott ("Pharm. Journ." [3], 51, 746) prefers the following modification of this process:—

Ten grms. of the opium in powder are exhausted with spirit of

·920 specific gravity. One or two drops of solution of ammonium oxalate are added, and then ammonia, until the liquid is only slightly acid. The spirit is now evaporated to one-third of its original volume, allowed to cool, and filtered. The filtrate is concentrated to about 5 c.c., transferred to a small flask, 4 c.c. of water and 3 c.c. of methylated spirit being used to wash the capsule. 2·2 c.c. of solution of ammonia (·960 specific gravity) are then introduced, 25 c.c. of ether being introduced at the same time. The flask is now closed with a well-fitting cork and shaken so as to mix the contents. After eighteen hours the ether is decanted as completely as possible, the precipitate collected on counterpoised filters, and washed with morphinated water. It is then dried, washed with benzene, dried, and weighed, and finally titrated with $\frac{N}{10}$ sulphuric acid. One c.c. of the acid = ·0303 grm. of

hydrated morphine. Although it is not essential, it is preferable to weigh the morphine before titrating, as an idea is thereby given of the amount of acid which will be required. This process is only recommended where the morphine is to be titrated.

Schidrowitz ("Analyst," xxix. 144) has devised a process which gives very constant results, and which is carried out as follows:—

Six grms. of opium roughly powdered are weighed into a small porcelain dish, 6 c.c. of distilled water are added, and the whole allowed to stand for about fifteen minutes. The contents of the dish are then worked up to a homogeneous consistence with a pestle, and then transferred (by means of successive small quantities of water) to a 100 c.c. tared Erlenmeyer flask. The total weight of opium and water is then made up to 54 grms. The flask, after corking, is shaken vigorously for five minutes, and is then allowed to stand for one hour, with an occasional shaking. The contents are then filtered through a filter, 10 cm. in diameter, into a second tared 100 c.c. Erlenmeyer flask. If the filtrate does not run clear at first it must be returned. When exactly 42 grms. of filtrate have been collected filtration is stopped. Next, to the 42 grms. of filtrate, exactly 2 grms. of a 50 per cent solution of salicylate of soda in water is added; the whole is then shaken for about half a minute, and immediately filtered as before. Of the filtrate 36 grms. are collected, and to this is added 15 c.c. of ether, and, after rotating the flask once or twice, 5·2 c.c. of a solution of ammonia, prepared by mixing 17 grms. of ammonia (specific gravity 0·960) with 83 grms. of water. The whole is then vigorously shaken for ten minutes, and the flask and contents are subsequently kept for twenty-four hours at a temperature of 12° C. After this, as much of the ether as is possible is poured off through a filter of 8 cm. in diameter, 15 c.c. of fresh ether is run into the flask, the latter rotated briskly (but so as to avoid forming an emulsion), and the ether again poured off through the filter. The whole of the liquid is then poured through the filter, the greater part (roughly two-thirds) of the crystals, however, being retained in the flask. The flask and filter are then washed with three lots each of 5 c.c. of water saturated with ether, and delivered from a pipette. Of each 5 c.c., 3 c.c. should be used to rinse the flask, and 2 c.c. run

directly on to the filter. The filter with its contents is removed from the funnel, folded, and gently but firmly pressed between sheets of filter-paper. The filter is then opened, and the greater part of the crystals returned to the flask. Filter and flask are then placed in an air oven at 55° C. until dry. It is then perfectly easy to transfer the small quantity of crystals still adhering to the filter to the flask.

Subsequently the crystals are dissolved in 25 c.c. $\frac{N}{10}$ H_2SO_4 , and

the excess of acid titrated with $\frac{N}{10}$ alkali, using methyl-orange as an indicator. It is preferable, prior to this titration, to dilute the liquid to roughly 50 c.c., and to fix the end-point by means of the droplet method. The percentage of morphine in the sample is then calculated as follows:—

Let x = number of c.c. $\frac{N}{10}$ acid employed, then $x \times 0.7575 + \frac{1}{13}$
 $(x \times 0.7575)$ = per cent morphine.

Nagelvort ("American Journal of Pharmacy," November 1900) has devised the following rapid process which gives fairly accurate results. Ten grms. are dried at 100° for three hours and powdered. The powder is transferred to a suitable filter and a mixture of 10 c.c. of ether and 10 c.c. of chloroform poured over it. The filter is covered and allowed to drain, and then 10 c.c. of chloroform poured on to it. After draining, it is dried by exposure to warm air and the powder is transferred to a flask holding about 120 c.c. To this, 100 c.c. of water are added. The flask is corked and well shaken at frequent intervals during two hours. Fifty c.c. is now filtered off and shaken with 10 c.c. of 95 per cent alcohol, 20 c.c. of ether and 1 c.c. of 10 per cent ammonia water. It is then allowed to stand for six hours. The precipitated morphine is collected on a tared filter, washed with morphinated water, pressed between filter paper, dried at 100° and weighed. The weight multiplied by 20 gives the percentage of morphine. It is to be noted, however, that no correction is made for the increase in volume of the 100 c.c. of liquid, due to the soluble portion of the opium.

PREPARATIONS OF OPIUM.

Extract of Opium is a semi-solid extract made by exhausting opium with water. Analysed by the official method described under opium, it should yield 20 per cent of morphine; 7 grms. of the extract should be used for the assay.

Liquid Extract of Opium.—This preparation is made by dissolving the extract in water and adding alcohol. It is a deep-coloured liquid having a specific gravity 0.985 to 0.995 (official), and when assayed for morphine, as described under tincture of opium, should contain between 0.7 and 0.8 gm. of morphine in 100 c.c. Twenty per cent by volume of 90 per cent alcohol is used in its preparation. The final product should contain 18 per cent of alcohol by volume. The solid residue averages 3 to 3.1 per cent.

Tincture of Opium.—This preparation is of the same alkaloidal strength as the liquid extract, but contains more alcohol. It is made with 45 per cent alcohol, but owing to the extractive matter, and allowing for slight loss during manufacture, the finished tincture should contain from 42 to 44 per cent of alcohol; no standards, other than the morphine content, are given in the Pharmacopœia. The extractive matter averages from 3·4 to 3·7 grms. per 100 c.c. The specific gravity varies from 0·955 to 0·962. The morphine should be between 0·7 and 0·8 grm. per 100 c.c., when assayed by the following process.

Pour 80 c.c. of the liquid into a porcelain dish; evaporate on a water bath until the volume is reduced to 30 c.c.; mix the residual liquid in a mortar with 3 grms. of freshly-slaked lime; dilute the mixture with water to 85 c.c.; set aside for half an hour, stirring occasionally. Filter off 50 c.c. of the liquid (representing 50 c.c. of the tincture) through a plaited filter having a diameter of about 1 decimetre, into a wide-mouthed stoppered bottle, having a capacity of 200 c.c.; add 5 c.c. of alcohol (90 per cent) and 30 c.c. of ether; shake the mixture; add 2 grms. of ammonium chloride; shake well and frequently during half an hour; set aside for twelve hours for the morphine to separate. Counterbalance two small filters; place one within the other in a small funnel in such a way that the triple fold of the inner filter shall be superposed upon the single fold of the outer filter; wet them with ether; remove the ethereal layer of the liquid in the bottle as completely as possible by means of a small pipette, and transfer it to the filter; pour into the bottle 15 c.c. of ether; rotate the contents and set the bottle aside; transfer the separated ethereal layer carefully, by means of the pipette, to the filter; wash the filter with a total amount of 10 c.c. of ether added slowly, and in portions; let the filter dry in the air; pour upon it the liquid in the bottle, in portions, in such a way as to transfer the granular crystalline morphine as completely as possible to the filter. When all the liquid has passed through, wash the remainder of the morphine from the bottle with morphinated water, until the whole has been removed. Wash the crystals with morphinated water until the washings are free from colour; allow the filter to drain; dry it, first by gentle pressure between sheets of bibulous paper, afterwards at a temperature between 131° and 140° F. (55° and 60° C.), finally at 230° F. (110° C.) for two hours. Weigh the crystals in the inner filter, counterbalancing by the outer filter. Take 0·3 grm. of the crystals, and titrate with decinormal volumetric solution of sulphuric acid, as directed under opium.

Dowzard ("Pharm. Jour.," [4] 17, 908) has pointed out that in the B.P. method for determining the morphine in the tincture a serious mistake has been made. Eighty c.c. of tincture and 3 grms. of slaked lime are used, and the mixture made up to 85 c.c. This is a very grave blunder, as the volume should only be made up to 81·9 c.c. Three grms. of slaked lime displace 1·44 c.c. of water.

Linimentum Opii.—This preparation contains half the quantity of morphine that the tincture does. It should contain about 55 per cent of alcohol.

Ammoniated Tincture of Opium.—This is a weak tincture of opium, and should contain from 0.1 to 0.12 gm. of morphine in 100 c.c. The specific gravity of properly prepared tinctures varies from 0.894 to 0.901. It should contain 2.06 grms. of benzoic acid per 100 c.c. which may be determined as described under paregoric. The solid residue varies from 2.7 to 2.9 grms. per 100 c.c.

Compound Tincture of Camphor, or Paregoric.—This preparation is a mixture of tincture of opium, benzoic acid, camphor, aniseed oil, and diluted alcohol. It should officially contain practically 0.46 mg. of anhydrous morphine per c.c. but otherwise no official tests are given. The average specific gravity of this tincture lies between 0.913 and 0.923, and the amount of extractive, dried at 105°, 0.3 to 0.37 gm. per 100 c.c. The alcohol content varies from 57 to 59 per cent by volume. The amount of morphine present may be determined by using 250 c.c. of the tincture, evaporating to 6 c.c. and then continuing the official process described under tincture of opium using one-fifth of the quantities throughout. It is to be noted that the statement that the tincture should contain 0.46 mg. of morphine per c.c. which appears in the Pharmacopœia is incorrect, since the official tincture of opium from which it is prepared is allowed to contain from 0.7 to 0.8 per cent, so that the proper limits for this preparation are 0.43 to 0.49 mg. per c.c.

Bird recommends the following as the best process for the detection of morphine in this tincture.

Compound tincture of camphor, 10 c.c. Evaporate to dryness on a water bath, take up with dilute alcohol and a minute drop of acetic acid, evaporate again to dryness, and dissolve the residue in 2 c.c. distilled water. One drop of this solution tested with Mayer's solution should give a copious precipitate.

Filter the aqueous solution and wash filter with distilled water. Transfer to a small separator and extract with hot amylic alcohol and a few drops of a saturated solution of potassium carbonate. Separate the amylic alcohol and wash the same with a half c.c. distilled water. Repeat the amylic extraction twice and evaporate the mixed amylic extracts on a water bath to dryness.

The amylic residue from a genuine tincture is at this stage brownish-yellow, but if no opium is present, nearly colourless.

Dissolve the amylic residue in 2 c.c. distilled water and four drops of diluted hydrochloric acid. Filter the solution through a small filter, with a little French chalk to remove colour, until perfectly bright, and wash filter with distilled water. Extract the clear aqueous solution in a separator with 4 c.c. hot amylic alcohol and sufficient powdered ammonium bicarbonate to make alkaline and repeat the extraction twice with successive 2 c.c. quantities of hot amylic alcohol. The mixed amylic extracts should be quite colourless and measure 8 c.c. Evaporate 2 c.c. of the amylic extract to dryness in a very small glass basin, concentrating the residue on to one spot, place on a white surface and moisten the residue with a very dilute solution of neutral ferric chloride. A perfectly distinct dirty blue coloration characteristic of morphine should appear. Another 2

c.c. evaporated should afford an orange-yellow colour with nitric acid.

The reactions may be compared with those obtained from 10 c.c. of a known sample of Tr. Camph. Co. carried through the process at the same time, when there should be no difficulty in coming to a conclusion as to the approximate correctness or otherwise of any sample in question. The reactions are also given quite distinctly with the residue from 2.5 c.c. tincture, but when that amount is taken one-fourth only of the quantities of solvent, etc., mentioned in the process must be used throughout.

It is very important that the amylic alcohol be specially redistilled; 20 or 30 c.c. evaporated in a glass capsule on the water bath should not leave the slightest residue.

The presence of the proper proportion of alcohol, which is from 57 per cent to 59 per cent by volume, is practically safeguarded by the specific gravity, which should lie between 0.913 and 0.923.

The benzoic acid may be determined by rendering 25 c.c. alkaline with soda, evaporating to 10 c.c., and extracting the last traces of camphor and aniseed oil with ether. The separated aqueous liquid is acidified with hydrochloric acid and extracted twice with ether. If the washed separated ethereal solution be allowed to evaporate spontaneously the benzoic acid may be weighed; or better, dissolved in excess of decinormal alkali, and the excess titrated with standard acid. Each 1 c.c. of decinormal alkali is equivalent to 12.2 mg. of benzoic acid.

A qualitative test for the presence of tincture of opium in this preparation is the reaction produced by the meconic acid always present in opium, when treated with ferric chloride. The liquid is diluted with 60 per cent alcohol until it is of a pale yellow colour, and a drop or two of ferric chloride solution added. A more or less deep red colour, due to meconate of iron, is produced.

Allen and Scott Smith ("Analyst," xxvii. 350) recommend the following process for detecting opium in such preparations as paregoric or cough mixtures. If 25 c.c. of the liquid be rendered alkaline with caustic soda, and evaporated to about 10 c.c., the alcohol and a portion of the camphor and oil of anise if present will be volatilized, and the amount of alcohol can be deduced with sufficient accuracy from the specific gravity of the distillate. On shaking the residue with ether, the remaining camphor and oil of anise will be extracted. If the ether be separated, and the aqueous liquid acidulated with hydrochloric acid, benzoic acid will in some cases be separated; but whether it separates or remains in solution it can be dissolved out by agitating the acidified liquid with ether. On allowing the separated ethereal solution to evaporate spontaneously in a small beaker, the benzoic acid is obtained in a state fit to weigh; but a better and more rapid plan is to repeatedly agitate the ethereal liquid with water until the washings no longer redden litmus, add a little more water and a few drops of phenol-phthalein solution, and titrate the liquid with $\frac{N}{20}$ caustic alkali (preferably baryta-water), which should be added

until the aqueous layer acquires a pink colour, not destroyed by agitation with the ether. Each 1 c.c. of $\frac{N}{20}$ alkali required represents 0.0061 grm. of benzoic acid. If 25 c.c. of the liquid has been employed, the number of mgs. of benzoic acid found, multiplied by 0.35, gives the grains of benzoic acid per pint of the liquid.

The detection of meconic acid proves the presence of opium in the tincture. When this information alone is sought the liquid may be diluted in a test-tube with 60 per cent alcohol till it is of a light yellow colour, and a drop or two of solution of ferric chloride then added. If opium be present, a more or less deep red coloration will be produced, owing to the formation of iron meconate. By comparing the depth of red colour with that given by a standard tincture a rough indication of the amount of opium present may be obtained.

Unmistakable confirmatory evidence of the presence of morphine in cough mixtures may be obtained by obtaining a microscopic preparation of its typical crystals in the following manner: A portion of the amylic alcohol alkaloidal extract is shaken out with a little dilute acetic acid, a few drops of the aqueous acetate solution are put in a watch glass or a celled microscope slide, covering it with another watch glass moistened with strong ammonia, and allowing to stand for half an hour. If morphine be present, the characteristic elongated prisms of the crystalline base will be detected on examining the liquid under the microscope.

The remaining galenical preparations of opium need not be discussed here, as they present no special individual features.

Morphine $C_{17}H_{19}NO_3 \cdot H_2O$ is the principal alkaloid of opium, as mentioned above. It is only official in the British Pharmacopœia in the form of three of its salts, the acetate, hydrochloride and tartrate.

It crystallizes in fine colourless trimetric prisms, containing one molecule of water, which is lost slowly at 90° to 100° . It dissolves in about 33,000 parts of water at 0° ; 4500 parts at 10° and about 2500 parts (according to Dott) at 15° . In boiling water it is soluble to the extent of about 1 in 400. It dissolves in 100 to 150 volumes of 90 per cent alcohol at 15° . It is very slightly soluble in chloroform, ether, and benzene. The solutions are alkaline and slightly laevorotatory. When pure it yields practically no colour with cold sulphuric acid; with nitric acid an orange-red colour changing to yellow is produced. Sulphuric acid containing 0.4 per cent of formaldehyde gives a purple colour. A solution in very dilute acids gives a copious precipitate with potassio-mercuric iodide and other alkaloidal precipitants. Traces of morphine mixed with a solution of starch and evaporated give a blue coloration with a spot of iodic acid. If a solution of morphine be heated with an aqueous solution of potassium ferrocyanide containing a drop of neutral ferric chloride solution, a deep blue colour is developed, and on standing, a blue precipitate.

The following are the official salts of morphine:—

Morphine acetate $C_{17}H_{19}NO_3 \cdot C_2H_3O_2 \cdot 3H_2O$, contains about 71.7 per cent of alkaloid. It is required to have the following characters by the British Pharmacopœia:—

A white crystalline or amorphous powder, almost entirely soluble in two and a half parts of water and in about 100 parts of alcohol (90 per cent). It loses acetic acid when exposed to the air. It affords the reaction for morphine mentioned under "*Morphinæ Hydrochloridum*" and the reactions characteristic of acetates. Two grms. of the salt form with 6 c.c. of warm morphinated water a slightly turbid solution, which is rendered clear by the addition of 0.1 c.c. of acetic acid, and this solution, when mixed with solution of ammonia in slight excess, yields a precipitate which, after washing and drying, as described under "*Morphinæ Hydrochloridum*," weighs 1.42 grms. If the salt yield a larger proportion of morphine than this, it should be recrystallized from hot water acidulated with acetic acid. Heated to redness with free access of air, it leaves no residue (absence of mineral impurities).

Morphine hydrochloride $C_{17}H_{19}NO_3$, HCl, $3H_2O$, contains about 76 per cent of alkaloid and is officially required to have the following characters:—

Acicular prisms of a silky lustre, or a white powder consisting of minute cubical crystals, unchanged by exposure to the air. Soluble in 24 parts of cold water, 1 part of boiling water, and in 50 parts of alcohol. It should be without action on litmus. Solution of ammonia causes a white precipitate in the aqueous solution with difficulty soluble in excess; solution of potassium hydroxide a similar precipitate readily soluble in excess. This precipitate yields mere traces to benzol (absence of other alkaloids). Moistened with nitric acid the salt yields an orange-red coloration; with test solution of ferric chloride a dull greenish-blue coloration. Heated on a water bath for ten or fifteen minutes with a few drops of sulphuric acid, cooled, and treated with a few drops of diluted nitric acid, it gives a violet colour rapidly passing to blood-red. It dissolves without coloration in strong sulphuric acid; the addition of a small quantity of sodium arsenate to a portion of this solution causes a bluish-green coloration, and a small quantity of bismuth oxyhydrate added to another portion gives a purplish-brown coloration. It affords the reactions characteristic of hydrochlorides. Two grms. of morphine hydrochloride dissolved in 250 c.c. of warm morphinated water, with solution of ammonia added in the slightest possible excess, will give on cooling a crystalline precipitate which, when washed with a little cold morphinated water and dried, should weigh 1.51 grms. The drying should be accomplished, first by pressing the precipitate between sheets of bibulous paper, then by exposing it to a temperature between 131° and 140° F. (55° and 60° C.), and finally to a temperature of 230° F. (110° C.) for twenty minutes. Heated to redness with free access of air, it burns, leaving no residue (absence of mineral impurities).

Morphine tartrate $(C_{17}H_{19}NO_3)_2$, $C_4H_6O_6$, $3H_2O$, contains about 74 per cent of alkaloid, and is officially required to have the following characters:—

A white powder consisting of fine nodular tufts of minute acicular crystals. Efflorescent at 68° F. (20° C.). Soluble in 11 parts of cold

water, almost insoluble in alcohol (90 per cent). It affords the reactions characteristic of morphine and of tartrates. Two grms. dissolved in 20 cubic centimetres of warm morphinated water, with solution of ammonia added in the slightest possible excess, will give, on cooling, a crystalline precipitate, which, after washing and drying as described under "*Morphinæ Hydrochloridum*" should weigh 1.47 grms. Heated to redness with free access of air, it burns without leaving any residue (absence of mineral impurities).

The Detection of Morphine.—In the solid state morphine is readily identified by the following reactions:—

A minute fragment, moistened with a drop of neutral* solution of iron alum, gives a characteristic greenish-blue colour, which is destroyed by free acids or by heat. Contact with strong nitric acid gives an orange-red colour, changed to yellow on standing, and destroyed by the addition of a few drops of solution of sodium thiosulphate. If a fragment be dissolved in sulphuric acid and a few drops of a solution of sodium or ammonium molybdate be added, a fine violet colour is produced, changing to blue-green and finally disappearing. This reaction is best applied by dissolving 5 mgs. of molybdate of ammonium in 1 c.c. of strong H_2SO_4 (Fröhde's reagent) and adding a few drops of this to the solid morphine. If a fragment be dissolved in H_2SO_4 , heated to 100° and a fragment of potassium perchlorate added, a deep red-brown colour is produced which rapidly spreads through the liquid. This is a very characteristic reaction. If a fragment of morphine be mixed with a little cane sugar, a drop of concentrated sulphuric acid will produce a beautiful purple colour. If a solution of morphine be saturated with sugar and the liquid poured into concentrated H_2SO_4 a purple or rose-red colour appears at the junction of the liquids. The addition of a drop of bromine water after the sulphuric acid increases the delicacy of this reaction. A fragment moistened with sulphuric acid containing 0.4 per cent of formaldehyde gives a fine purple colour.

Morphine in solution is precipitated by alkalis or alkaline carbonates but is redissolved by excess, except by alkaline bicarbonates. In solutions free from interfering substance, morphine can be precipitated by sodium bicarbonate, collected, washed with morphinated water, and weighed, and thus quantitatively determined. In complex solutions morphine is liberated from its salts by the use of an alkali, and the solution well shaken for a long time with hot amyl alcohol. The solvent should first be added and then the alkaline bicarbonate, as, if the morphine be allowed to crystallize, it is very difficult to dissolve in the solvent. After separation, if a determination be required, the process should be repeated three times, and the amyl alcohol evaporated at 100° , and the morphine weighed or titrated with dilute sulphuric acid using methyl-orange as indicator. Or, better, the amyl alcohol is repeatedly shaken with very dilute hydrochloric acid, and the acid solution precipitated by sodium bicarbonate. If only a quantitative reaction is desired, the hydrochloric acid solution is concentrated and a few drops of a mixture of ferric chloride and potassium ferri-cyanide added, when prussian blue is formed. A solution of iodine in hy-

driodic acid gives a crystalline precipitate in very dilute solutions of morphine. A very delicate test, but one which is merely confirmatory, since many other bodies give it—is as follows: If a dilute solution of morphine be mixed with a few drops of starch solution and evaporated to dryness, the residue, moistened with iodic acid, will give a blue colour if as little as $\frac{1}{200000}$ of a grain of morphine be present.

Codeine. This alkaloid, $C_{17}H_{18}(CH_3)NO_3 \cdot H_2O$, is methyl-morphine and may be obtained from opium, or by the methylation of morphine. It is thus described in the British Pharmacopœia:—

In colourless or nearly colourless trimetric crystals soluble in 80 parts of water or of solution of ammonia, readily soluble in (90 per cent) chloroform, and in diluted acids. It is soluble in 30 parts of ether. The aqueous solution has a bitter taste and an alkaline reaction. The alkaloid dissolves in an excess of sulphuric acid, forming a colourless solution, a small quantity of which, when gently warmed on a water bath with 2 drops of solution of ammonium molybdate, or with a trace of ferric chloride or potassium ferricyanide, develops a blue or bluish-black colour, which on the addition of a minute trace of diluted nitric acid, changes to a bright scarlet, becoming orange. Heated to redness in air it yields no ash. Moistened with nitric acid the liquid becomes yellow but not red. A 2 per cent solution of codeine in water acidulated with a few drops of hydrochloric acid, gives a whitish precipitate with solution of potassium hydroxide, but not with solution of ammonia. A saturated solution of codeine in water acidulated with hydrochloric acid, should give no blue colour, but only gradually a dull green, on the addition of test solution of ferric chloride and a very dilute solution of potassium ferricyanide (absence of morphine and other impurities).

[It is to be noted that codeine frequently gives a green colour with cold sulphuric acid. This, however, is due to the presence of a trace of selenium as an impurity in the acid.]

Codeine is sharply differentiated from morphine by its ready solubility in ether and chloroform, by which solvents it can be extracted from its solutions when rendered alkaline. When warmed with sulphuric acid, codeine (and other bodies also) gives a blue colour, in the presence of a trace of any oxidizing agent such as arsenic acid. A fragment, treated with two drops of sodium hypochlorite solution and four drops of sulphuric acid, gives a fine blue colour.

Claassen ("Jour. Chem. Soc." 58, 1198) proposes estimating codeine when it exists in the free state in neutral solutions, by allowing it to decompose morphine sulphate, to a solution of which it is added. The precipitated morphine, multiplied by 0.9868 represents the codeine.

Codeine phosphate ($C_{17}H_{18}[CH_3]NO_3 \cdot H_3PO_4)_2 \cdot 3H_2O$, is thus described in the British Pharmacopœia:—

White crystals which have a slightly bitter taste. It is soluble in 4 parts of water, much less soluble in alcohol (90 per cent). A 5 per cent aqueous solution has a slightly acid reaction, and yields a whitish precipitate with solution of potassium hydroxide, but not with solution of ammonia. It affords the reactions characteristic of codeine

and of phosphates. It loses its water of crystallization when dried at 212° F. (100° C.), and at a higher temperature melts, forming a yellowish-brown liquid. It should yield no characteristic reaction with the tests for chlorides or sulphates. It should not be coloured blue by test solution of ferric chloride (absence of morphine).

Apomorphine hydrochloride $C_{17}H_{17}NO_2 \cdot HCl$ is an alkaloid obtained by heating morphine or codeine hydrochloride in a sealed tube to 150° with excess of hydrochloric acid or with zinc chloride. Apomorphine hydrochloride is official in the British Pharmacopœia, which describes it as follows:—

Small, greyish-white, shining, acicular crystals, turning green on exposure to light and air. Inodorous. Soluble in 50 parts of water and more soluble in alcohol (90 per cent), the solutions being decomposed with production of a green colour when they are boiled. Neutral or very feebly acid to solution of litmus. From solutions, solution of sodium bicarbonate throws down a precipitate which becomes green on standing and then forms a solution which is purple with ether, violet with chloroform, and bluish-green with alcohol (90 per cent). With dilute test-solution of ferric chloride it gives a deep red, and with nitric acid a blood-red coloration. If the salt imparts an emerald-green colour to 100 parts of water, after shaking the mixture, it should be rejected.

A solution containing 1 part in 100,000 will yield a green coloration when rendered faintly alkaline with potassium bicarbonate and exposed to the air.

Dr. Hasse has shown that much of the so-called "Apomorphinum Hydrochloricum" on the market is not apomorphine hydrochloride, but a hydrochloride of trimorphine, and only contains traces of apomorphine. Frerichs has investigated this substance and found that the "apomorphine hydrochloride" in question did not conform to the German Pharmacopœia requirements owing to the fact that it is not crystalline, and instead of dissolving in 40 parts of water only its own weight of water was required for solution. Dr. Frerichs shows that as regards the other Pharmacopœia tests the spurious preparation does not differ noticeably from the authentic. In the case of pure apomorphine hydrochloride the solution darkens more rapidly with soda solution than in the case of the spurious hydrochloride, and the deposit obtained with sodium bicarbonate from a solution of true apomorphine hydrochloride quickly assumes a green shade, while this is hardly perceptible in the case of the spurious. A solution of the precipitated alkaloid in ether or chloroform is decidedly coloured in the case of the authentic apomorphine, whereas in the case of the spurious preparation it is scarcely tinged. Dr. Frerichs considers that the new edition of the Pharmacopœia should distinguish by tests between the trimorphine and apomorphine salts, and he gives the following test as suitable for the purpose:—

Place 10 cg. of the apomorphine hydrochloride on a small dry filter and pour over it 5 c.c. of a mixture of hydrochloric acid 1 part and water 4 parts. To the filtrate add potassio-mercuric iodide solution. Pure apomorphine hydrochloride gives at the most an

opalescent turbidity, but if other alkaloids are present the hydrochloric acid filtrate gives a distinct precipitate with potassio-mercuric iodide.

According to Harnack and Hildebrand, however, this impurity is probably β -chloromorphide, with, at most, traces of trimorphine.

As little as 10 per cent of trimorphine hydrochloride in the apomorphine salt is detectable by this test, as also are morphine and cinchona alkaloids.

PODOPHYLLUM.

The official drug of the Pharmacopœia is *Podophyllum peltatum*, which is used as the source of manufacture of podophyllum resin. It will be advisable, however, to discuss the allied drug, *Podophyllum emodi*, which will probably be rendered official in the next edition of the Pharmacopœia. The drug consists of the dried rhizome and roots. The resin is obtained by exhausting the powdered drug with 90 per cent alcohol, recovering the bulk of the alcohol and then precipitating the resin by pouring the remaining liquid into water rendered acid with HCl. The precipitated resin is collected and dried at a temperature not exceeding 38° C. It is described officially as soluble, or nearly so, in 90 per cent alcohol and in ammonia, but not in acid liquids. It should not yield more than 1 per cent of ash. [The root yields an average of 5 per cent of ash.] The value of the drug lies in the resinous matter present. Dunstan and Henry ("Proc. Chem. Soc." 1898, 189) have examined the constituents of *Podophyllum peltatum* (American podophyllum) and of *Podophyllum emodi* (Indian), and found them to be identical. The principal constituent is podophyllo-toxin, a neutral crystalline substance of the formula $C_{15}H_{14}O_6$, melting at 117°, first isolated by Podwyssozki and Kürsten. An uncrystallizable resin, podophylloresin is also present. The product known commercially as podophyllin is the mixed resinous matter of the drug. American podophyllum, the official variety, contains 4 to 5 per cent, whilst the Indian variety contains 9 to 12 per cent. According to Dunstan and Henry the crystalline podophyllotoxin is present to the extent of about 1 per cent in the American, and from 2 to 5 per cent in the Indian drug. According to Umney the following represents the average characters of the resins of the two drugs:—

	P. Emodi.	P. Peltatum.
	Per cent	Per cent
Resin, by official process for podophyllin resin	11·4	5·9
Constituents of the resin—		
Podophyllotoxin (crude)	17·8	33·8
Pure crystalline pieropodophyllin	2·6	4·5
Pieropodophyllic acid	not determined.	not determined.
Podophyllic acid	30·8	6·9
Podophylloquercetin	1·3	2·4
Fatty matter	2·3	5·7

Sample.	Description of the Resin.	P. c. of Ash.	P. c. Insoluble in Alcohol 90 p. c.	P. c. Insoluble in Solution of Ammonia.	P. c. Insoluble in P. c. Chloroform.	P. c. of Chloroform Extract Washed with Ether.	Character of the Same.	P. c. Insoluble in Pure Ether.	Character of the Residue.	P. c. of Ether Extract with Petroleum Ether.	Character of the Same.	General Remarks.
1	Very dull yellow.	0.4	0.2	—	45.3	46.6	Cream-coloured powder.	43	Sticky, drying to a dark resin.	43.3	Brittle brown mass.	The ash contained iron.
2	Dull light yellow.	0.4	2	4.4	34	53	"	30	"	60	A mixture of brownish masses and a yellow powder.	The ash contained copper.
3	Dull light yellow.	0.4	—	1	34	50.5	"	28.2	"	52.5	"	The ash contained copper.
4	Dull greyish-green.	0.4	2.2	6	36	47	"	35.2	"	44.5	"	The ash contained iron.
5	Deep bright yellow.	0.4	3	2.3	44.2	41	"	31	Powder with a slight admixture of resin.	53	Brittle brown mass.	—
6	Dark greenish-yellow.	0.4	1.3	—	33.3	39	"	—	—	—	—	The ash contained copper.
7	Dull greenish-yellow.	0.5	0.8	0	33.8	47.5	"	29.8	Powder with a slight admixture of resin.	47	A mixture of brownish masses and a yellow powder.	The ash contained iron.
8	Deep bright yellow.	0.6	2.4	3	42.6	46.6	"	35	Somewhat resinous but it did not form a mass.	53.5	"	—
9	Brown.	0.946	1.4	0.8	46.6	42.3	"	38	"	46.6	"	—
10	Greenish-yellow.	1.3	6	0.2	41.8	50	Brown mass.	30.2	Powder.	44.5	"	The ash contained iron.
11	Light bright yellow.	1.4	—	—	51.3	33.3	Cream-coloured powder.	—	—	—	—	—
12	Deep bright yellow.	1.6	10	1	53.3	39	"	40	Powder.	40	A mixture of brownish masses and a yellow powder.	—
13	Dull greenish-yellow.	5.19	12.2	3	62	37.5	"	49.2	"	42.5	"	—

Apart from the official tests given above, a genuine podophyllum resin should yield from 30 per cent to 40 per cent of podophyllotoxin, when examined in the following manner: The resin should be extracted with cold chloroform, and the bulk of the chloroform evaporated. The liquid remaining is poured into petroleum ether and the precipitate washed with petroleum ether, dried and weighed (Kremel, "Year-Book of Pharmacy," 1889, 180). The Indian resin is stated by Verney to contain a smaller percentage of podophyllotoxin.

About 50 per cent of the resin dissolves in chloroform.

Taylor ("Pharm. Journ." 4, 15, 368) has made a number of analyses of typical samples and considers that: (1) The ash limit of 1 per cent is reasonable (high ash values indicate the use of alum in precipitating the resin, in order to get it of good colour). (2) The solubility in ammonia is not of much value. (3) That at least 95 per cent should dissolve in 90 per cent alcohol. (4) That 50 per cent should be soluble in chloroform. (5) That at least 40 per cent of crude podophyllotoxin should be obtained by precipitation of the chloroformic solution with petroleum ether. (6) That 60 per cent should be soluble in pure ether, and that the residue should consist of a resinous and sticky substance.

The table on opposite page embraces Taylor's examination of thirteen typical samples.

Millard ("Pharm. Journ." 4, vi. 304) gives the following as a reliable means of discriminating between the American and Indian resins:—

0·4 grm. of resin is mixed with 3 c.c. of alcohol of specific gravity 0·920 and 0·5 c.c. of liquor potassæ. The test tube is shaken, and in the case of the Indian resin, the mixture becomes semi-solid, so that the tube can be safely inverted. If solidification does not take place at once, it will do so on boiling and then cooling the liquid. The American resin gives a dark fluid under the same conditions.

Tincture of Podophyllum is a solution of 320 grains of the resin in 90 per cent alcohol to make 1 pint. It should have a specific gravity from 0·844 to 0·848. If made with a well-prepared resin, it should yield 3·5 grms. of solid residue per 100 c.c., but many samples do not contain more than 3 grms. per 100 c.c. The amount of alcohol should not be below 86 per cent by volume.

RHUBARB.

The rhubarb official in the British Pharmacopœia is the rhizome of *Rheum palmatum*, *Rheum officinale*, and probably other species; collected in China and Tibet.

No standards of purity are given in the Pharmacopœia, other than external appearance.

Rhubarbs grown in Europe are usually from other species and are quite different in their constituents, and are therefore not official.

The constituents of rhubarb which are of importance have been

described from time to time by different chemists, and their descriptions are very discordant.

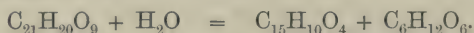
A careful examination of the most reliable researches shows that these discrepancies are probably due to the fact that rhubarb contains a very unstable substance which easily breaks down into simpler compounds.

This substance is known as rheopurgin, which decomposes into four glucosides.

These are bodies which yield a sugar on hydrolysis, and a derivative of anthraquinone in each case.

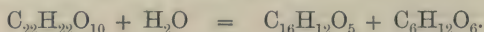
They are as follows:—

(1) Chrysophanein $C_{21}H_{20}O_9$, which yields chrysophanic acid on hydrolysis as follows:—



According to Gilson pure chrysophanic acid melts at 195° to 196° .

(2) Rheochrysin $C_{22}H_{22}O_{10}$, which yields rheochrysidin (formerly known as isoemodin or rhaberone) as follows:—



(3) A glucoside of unknown characters which readily yields emodin $C_{13}H_{10}O_6$ melting at 256° .

(4) Another unknown glucoside which readily yields rhein $C_{15}H_8O_6$ melting at 314° .

In addition to these bodies there are several astringent bodies of which several are glucosides yielding gallic acid on hydrolysis.

As this work is going to press, an important paper on this subject has just been read at the meeting of the Chemical Society held on 6th April, 1911, by Tutin and Clewer, to which reference should be made in the Society's Journal.

The analysis of rhubarb has been the subject of numerous experiments, but no process for the determination of the active principles can be said to be very satisfactory. The determination of the moisture and mineral matter and the microscopic examination are the most useful methods available, together with an approximate determination of the emodin and chrysophanic acid of the root and special tests for such adulterations as turmeric and added oil. A compound tincture is official. Its characters are given in the table on p. 496.

Moisture and Ash.—The moisture in normal powdered rhubarb does not exceed 8 per cent to 9 per cent, and the ash varies from 5 per cent to 12.5 per cent, the high ash being due to the presence of a considerable amount of calcium oxalate in the drug.

Extractive.—Genuine rhubarb should yield not less than 33 per cent of dry extractive matter to 45 per cent alcohol.

The Glucosides.—The separation of the glucosides, which is rarely necessary in actual practice, may be effected in the following manner: the rheopurgin is extracted by percolation with a mixture of 5 volumes of methyl alcohol and 95 volumes of ether. As extraction proceeds, the amount of methyl alcohol in the solvent liquid is gradually increased up to 40 per cent. The percolate is concentrated

when a yellow crystalline powder commences to be deposited. To avoid decomposition, the concentration must now go on in vacuo. The deposited yellow powder is washed with a mixture of methyl alcohol (1 volume) and ether (3 volumes), and finally with pure ether, and dried in vacuo at ordinary temperature. Twenty grms. of the crude substance are macerated for 5 days with 1 litre of 2 per cent sodium carbonate solution. It is then filtered and the insoluble portion again treated with 750 c.c. of the same solution for half an hour. After standing in a cool place for twenty-four hours it is filtered. The filtrates contain the rhein and emodin, and are washed. On acidifying with dilute H_2SO_4 and warming for a short time on the water bath, rhein and emodin are precipitated. From the washed and dried precipitate emodin is extracted by boiling chloroform, and rhein is extracted from the residue by boiling pyridine and recrystallized from methyl alcohol.

The portion insoluble in Na_2CO_3 solution is then digested at 70° for forty-five minutes with the same solvent, and filtered hot. After cooling the filtrate throws down a precipitate, which is collected, washed and dried, and kept separate. The filtrate is then acidified and the precipitate collected. The melting-points of the products of hydrolysis of the two precipitates are determined and those melting from 184° to 186° are put together; and those melting at 199° to 201° are put together; and each bulking is again treated with hot sodium carbonate solution as before, and so on until no further separation can be effected. The two bodies are recrystallized from 90 per cent alcohol. When the chrysophanein melts at 248° to 249° , and yields on hydrolysis chrysophanic acid melting at 193° . The rheochrysin yields rheochrysidin melting at 204° .

The determination of emodin (which is approximate only) is best determined by the following colorimetric method, the standard colour being that given by 0.001 gm. of pure emodin obtained from aloes, dissolved in 1 litre of water rendered slightly alkaline with KOH.

This has a pale rose colour. 0.5 gm. of rhubarb, in very fine powder, is boiled for fifteen minutes, under a reflux condenser, with 50 c.c. of 50 per cent H_2SO_4 ; the anthra-glucosides are thus hydrolysed and anthraquinone derivatives set free. When cold, the liquid, without filtration, is shaken out with successive 50 c.c. of ether until that solvent is no longer coloured and does not give a rose colour when a portion is tested with KOH. The separated aqueous liquid is again boiled for fifteen minutes, cooled, and again shaken out with ether. The bulked ether extracts are then shaken out with successive washings of 5 per cent KOH solution until a rose tint is no longer obtained. The bulked alkaline liquid is then made up to 500 c.c. One hundred c.c. of this solution is diluted to 1 litre; the colour is then matched against that of the standard emodin solution, on a white surface, in the usual manner. The tint of the rhubarb solution will generally be too dark; it must therefore be diluted with a known volume of water.

The method advocated by Tschirch and Edner ("Archiv der Pharm." 245, 150) for the approximate determination of chrysophanic

acid gives good results. From 0.5 gm. to 1 gm. of the rhubarb in fine powder is exhausted by boiling several times with 5 per cent alcoholic potash solution. The alkaline liquids are then distilled to remove nearly all the water, and the residue is slightly diluted with water and rendered acid with HCl. The precipitate is washed with slightly acidified water and dried, and then extracted with chloroform in a Soxhlet tube. The oxymethyl-anthraquinone derivatives are thus removed. The chloroform is recovered and the residue dissolved in 10 c.c. of 5 per cent solution of NaOH, and diluted with water to 50 c.c. A solution of p-diazonitraniline is prepared by shaking 5 grms. of p-nitraniline with 25 c.c. of water and a little strong sulphuric acid. Another 100 c.c. of water and 3 grms. of NaNO_2 in 25 c.c. of water are then added. The whole is then made up to 500 c.c. Twenty c.c. of this reagent are then added to the alkaline rhubarb extract and well shaken, and hydrochloric acid is added drop by drop until the red colour is discharged and an acid reaction obtained. The liquid is set aside for four hours, and the precipitate collected on a rated filter, washed with water, dried at 70° and weighed. 4.47 parts of the precipitate are representative of 2.54 parts of chrysophanic acid (or say $4.5 = 2.5$ parts). According to the most reliable results, the following are the amounts of chrysophanic acid and emodin present in various types of rhubarb:—

	Chrysophanic Acid.	Emodin.
	Per cent	Per cent
Chinese	2.5 to 4.3	1.8 to 2.8
English	1.5 „ 1.9	0.5 „ 1.5
French	1.0 „ 1.5	0.4 „ 1.5
Austrian	0.9 „ 1.6	0.5 „ 1.5

Oil.—Not more than 0.3 per cent of fat should be present in pure rhubarb; if, on extracting with ether in a Soxhlet tube, more than this be found, it is practically certain that a little oil has been added in order to improve the colour of the powder.

The Detection of Turmeric.—One gm. of powdered rhubarb is shaken for a few minutes with 10 c.c. of chloroform, and the mixture is filtered. The filtrate is agitated with 15 times its volume of petroleum ether and the mixture divided into 2 parts, one of which is shaken once or twice with 2 c.c. to 3 c.c. of pure strong sulphuric acid, while the other is shaken with 1 c.c. to 1.5 c.c. of saturated solution of borax. If the sample be pure, the original chloroform solution will show a pale, straw-yellow colour, which disappears on mixing with the petroleum ether. The treatment with sulphuric acid will impart a pale brown colour to the latter, while the supernatant liquid remains colourless. The treatment of the second portion with strong borax solution should produce no change in colour. If, however, the sample under examination was adulterated with turmeric, the following reactions will be obtained: the chloroform solution

will show a yellowish-brown colour and a well-marked greenish fluorescence. The addition of petroleum ether will cause the formation of a yellow flocculent precipitate in the chloroform solution, while the yellow colour of the liquid and the green fluorescence remain unchanged. The mixture of chloroform solution and petroleum ether, when shaken with sulphuric acid as stated, will change to violet, while the acid itself will assume an intense red coloration, changing rapidly to reddish-brown and then slowly to yellowish-brown. The agitation of the second portion with borax solution will cause the latter to turn violet, the upper layer remaining unchanged.

Microscopic Examination.—On examination under the microscope, characteristic star-like aggregations of calcium oxalate are to be seen, and small starch grains somewhat resembling those of the pea



FIG. 55.—Powdered rhubarb.

or bean, with a strongly marked hilum. Large reticulated vessels and thin parenchymatous cells containing a few starch grains are also to be found. Added starchy matter will be detected by the character of the starch grains. The presence of turmeric is indicated by irregular masses of gelatinized starch, and the universally distributed yellow colouring matter, changed to a deep red by sulphuric acid diluted with an equal volume of alcohol, in which the red
 pur dissolves.

STRAMONIUM.

Both the dried leaves and the dried ripe seeds of *Datura stramonium* are official in the Pharmacopœia, as well as a tincture of the former and a semi-solid extract of the latter. No standards are given for any of these.

The leaves contain about 0.25 (from 0.15 to 0.32 per cent) of alkaloids, consisting principally of hyoscyamine, with some atropine and hyoscyne. The seeds contain about the same amount of alkaloids and in about the same proportions. Hyoscyamine and atropine are described on pp. 520, 521; hyoscyne is described on p. 521.

The ash of stramonium leaves varies from 14 to 22 per cent; that of the seeds from 2 to 3 per cent.

The alkaloids may be estimated by exhausting the leaves or seeds with alcohol of 60 per cent strength and then using the process described under the tincture.

Microscopic Examination.—In powdered stramonium leaves, numerous fragments will be found which show partial sections of the



FIG. 56.—Powdered stramonium leaves $\times 240$. *cr*, crystals; *ccr*, crystal cells; *ei*, lower epidermis; *en*, neural epidermis; *es*, upper epidermis; *ffv*, debris of fibro-vascular bundles; *l*, bast; *me*, spongy parenchyma; *pa*, *p'a*, palisade-tissue; *pg*, glandular hairs; *po*, pollen grains; *pt*, simple hairs; *tf*, cortical tissue of midrib; *tr*, *v*, vessels, etc. (Greenish & Collin).

By permission of the Editor of the "Pharmaceutical Journal".

leaf, in which numerous rosettes of calcium oxalate are to be found. A few glandular hairs will be seen and a fair number of pitted and other vessels.

Tincture of Stramonium.—This is prepared by exhausting 4 ounces of the leaves with sufficient 45 per cent alcohol to give 20 fluid ounces of the tincture. It should have the following characters:—

Specific gravity	0.953 to 0.962
Solid residue	3.2 „ 4 grms. per 100 c.c.
Alcohol by volume	42 to 43 per cent
Alkaloids	0.02 to 0.03 per cent

The alkaloids are determined by the process devised by Farr and Wright ("Pharm. Journ." 3, xxii. 569) which is as follows:—

Fifty c.c. of the tincture to be estimated are introduced into a porcelain dish, and evaporated over a water bath to low bulk, water being added, if necessary, until all the spirit is removed. The residual liquor is allowed to cool, and is acidified with 1 c.c. of semi-normal sulphuric acid, and the liquid filtered through cotton-wool into a separator. The dish and filter are rinsed first with a little acidulated water, and then with 15 c.c. of chloroform, the rinsings added to the contents of the funnel, and the whole well shaken. After separation the chloroform is drawn off, and the process repeated with 10 c.c. of chloroform. The washings are mixed and freed from traces of alkaloid by shaking with three successive small portions of acidulated water, and these are separated and added to the original solution. The latter is then made alkaline with ammonia, and the alkaloids extracted with three successive quantities of chloroform of 15 c.c. each. To obtain the alkaloids in a pure condition, they are withdrawn from solution in chloroform by agitation with three successive small portions of acidulated water, the mixed acid solutions made alkaline with ammonia, and the alkaloids taken out by agitation first with 10 c.c., and then with two successive quantities of chloroform of 5 c.c. each. In cases where the final acidified aqueous solution is not colourless, the process of shaking out is repeated. The mixed chloroformic alkaloidal solutions are afterwards shaken with ammoniated water, and after separation are drawn off and evaporated over a water bath, and the alkaloidal residue heated at 100° until the weight is constant.

If the alkaloids are titrated, which is perhaps, the more correct method, as in the case of gelsemium, 1 c.c. of $\frac{1}{20}$ th normal HCl is equivalent to 0.01445 gm. of alkaloid.

STROPHANTHUS.

The dried ripe seeds of *Strophanthus kombé* are official in the Pharmacopœia, as well as an extract and a tincture, but no standards are given for them, other than the following colour test which is intended to distinguish the *kombé* seeds from those of other species.

The thin endosperm surrounding the cotyledons of the seed is coloured dark green by sulphuric acid (presence of strophanthin).

The principal constituent of the seeds is the glucoside strophanthin $C_{40}H_{66}O_{19}$, which is present to the extent of from 1.8 to 3.2 per cent.

Seeds from other varieties of *strophanthus* are frequently present in commercial parcels of the drug and it is not easy to distinguish between them and the *kombé* seeds.

According to Gordon Sharp ("Pharm. Journ." 4, xxiii, 258) the official test with sulphuric acid is not always reliable and should be modified as follows: a seed should be cut into four pieces and placed on a white dish in which are 20 drops of 13.6 per cent sulphuric acid. Allow to stand for one minute. The dish is then rotated over a Bunsen flame and in half a minute, the dark green colour will appear at the edge of the fluid if the seeds are genuine. The green colour rapidly spreads and if heating be continued, a red, and finally black colour appears.

E. M. Holmes recommends the use of cold 80 per cent sulphuric acid.

The seeds should yield from 3.5 to 4.5 per cent of ash on incineration. The most reasonable method of valuing the seeds appears to be that of Barclay ("Pharm. Journ." 4, iii, 463). Twenty grms. of the seeds in coarse powder are extracted with carbon bisulphide in order to remove the fat. The seeds are then exhausted with 70 per cent alcohol, in a Soxhlet tube, and the alcoholic liquid diluted with its own volume of water and the alcohol evaporated. The filtered aqueous liquid is then digested for an hour on the water bath with 1 per cent of sulphuric acid. This results in the hydrolysis of the strophanthin with the formation of strophanthidin $C_{26}H_{38}O_7$. This can be extracted by shaking with three successive quantities of warm amyl alcohol, the solvent evaporated and the residue weighed. One part of strophanthidin is equivalent to 1.84 parts of strophanthin.

Mann ("Year-Book of Pharmacy," 1906, 249) has recorded much higher figures for strophanthin in *strophanthus* seeds, but they appear to lack confirmation. Cæsar and Loretz (Report, September, 1905) give the following method for the assay of the seeds:—

Seven grms. of crushed *strophanthus* seeds are treated in a flask with 70 grms. of absolute alcohol, and the gross weight noted. The whole is then digested, under a reflux condenser, on the water bath for one hour. When cold the original weight is made up by the addition of more absolute alcohol, and 50.5 grms. are filtered off (=5 grms. of seeds). The solvent is then evaporated, and the alcohol-free residue treated with petroleum ether, to remove the fat, the solution being passed through a small filter. The insoluble residue on the filter is then washed back into the rest, in the capsule, with 5 to 8 c.c. of boiling water. The whole is then heated to boiling and treated with 5 drops of basic lead acetate solution. The precipitate is collected on a filter, and washed with boiling water until the filtrate is free from bitterness. This aqueous filtrate is boiled and freed from excess of Pb by means of SH_2 , the PbS being filtered off. On evaporating an aliquot part of this filtrate, the residue may be weighed, when dry, as crude strophanthin. To determine the amount of pure strophanthin, the above aqueous filtrate is hydrolysed by boiling for two hours with 5 drops of pure HCl. When the volume of liquid is reduced to 10 c.c., it is made up to 20 c.c. with water, and, when cold, shaken out

with successive washings of CHCl_3 , the CHCl_3 extracts being bulked in a small tared flask. The aqueous portion, after shaking out, is again boiled for thirty minutes, and again shaken out with CHCl_3 , the process being repeated as long as any bitter taste is evident. The bulked CHCl_3 solutions are then distilled to dryness and the residue, when constant, weighed as strophanthidin. The product $\times 1.84$ gives the equivalent of strophanthin.

As has been pointed out by Gilg, Thoms and Schedel ("Berichte Pharm." 14, 90) the various species of strophanthus yield glucosides which are not identical, hence any attempts at standardization, must, to be of value, have reference to the botanical origin of the seeds.

Tincture of Strophanthus.—This is prepared by exhausting half an ounce of the seeds with sufficient 70 per cent alcohol to produce 1 pint of tincture. A genuine tincture should have the following characters:—

Specific gravity	:	.	.	.	0.894 to 0.897
Solid residue	0.4 „ 0.7 gm. per 100 c.c.
Alcohol by volume	68.5 „ 69 per cent
Strophanthin	0.05 „ 0.08 „

The strophanthin may be determined by evaporating 100 c.c. of the tincture, diluted with an equal volume of water, until the alcohol is removed and then proceeding as with the assay of the seed.

Extract of Strophanthus.—This is an official semi-solid extract containing the active principles of half its weight of the seeds. Ten grms. should be rubbed down with 70 per cent to a cream and then warmed for an hour with about 50 c.c. of 70 per cent alcohol. On cooling it is filtered, the filter washed well with more 70 per cent alcohol, and the strophanthin estimated as in the case of the tincture. Three authentic samples made from *kombé* seeds gave the following results: 0.9 per cent; 1.15 per cent; 1.26 per cent. About 1 per cent to 1.3 per cent should be found in well-made extracts.

CHAPTER X.

THE ESSENTIAL OILS OF THE BRITISH PHARMACOPŒIA.

1. OLEUM ANETHI.

DILL oil is the product of the distillation of the fruit of *Anethum graveolens*.

The British Pharmacopœia describes this oil as follows:—

“The oil distilled from Dill fruit.

“*Characters and Test.*—Colour pale yellow, odour that of the fruit, taste sweet and aromatic. Specific gravity 0.905 to 0.920. It rotates the plane of a ray of polarized light not less than 70° to the right, at 60° F. (15.5° C.), in a tube 100 millimetres long.”

The British Pharmaceutical Codex describes the oil as having a specific gravity 0.905 to 0.915 and an optical rotation of $+75^\circ$ to $+80^\circ$.

The oil is also distilled from a plant grown in India which is probably *Anethum sowa*. The oil from the European plant is a pale yellow liquid, which sometimes has a specific gravity as low as 0.895 but which then is of too low a standard to be used in medicine. The oil from the Indian plant usually has a specific gravity of 0.945 to 0.970 and an optical rotation from $+40^\circ$ to $+50^\circ$.

It is the oil from the European plants which is official in medicine in this country. This oil has a refractive index of about 1.4900. It consists almost entirely of carvone and limonene. The oil should possess the characters above given, and on distillation not more than 15 per cent should distil below 185° and not less than 40 per cent above 220° . The carvone may be estimated by the process described under oil of cinnamon.

2. OLEUM ANISI.

This oil is either distilled from the true aniseed, *Pimpinella anisum* or from the star aniseed, *Illicium verum*. The latter plant is cultivated in Southern China and Tonkin and furnishes the greater portion of the aniseed oil of commerce.

The British Pharmacopœia describes this oil as follows:—

“The oil distilled from Anise fruit; or from the fruit of the star anise, *Illicium verum*, Hook. fil. [“Bot. Mag.” plate 7005].

“*Characters and Tests.*—Colourless or pale yellow; with the odour of the fruit, and a mildly aromatic taste. It congeals, when stirred, at temperatures between 50° F. and 59° F. (10° C. to 15° C.) and should not again become liquid below 59° F. (15° C.). Specific gravity—at 68° F. (20° C.)—0.975 to 0.990. It rotates the plane of a ray of polarized light slightly to the left.”

It is a pale yellow oil of a syrupy consistence. The specific gravity lies between 0.975 and 0.990 at 20°. The rotation varies between + 0° 30' and - 2°. It is soluble in three volumes of 90 per cent alcohol. The refractive index varies from 1.5520 to 1.5600. The usual adulterants of this oil are petroleum, fennel oil, and the waste liquid portion of aniseed oil obtained in the manufacture of anethol. The value of the oil depends upon the quantity of anethol it contains, and as this melts at 21° to 22° and boils at 232° the melting-point and behaviour on distillation furnish valuable information as to the value of the oil. Not less than 80 per cent should distil between 225° and 235°.

Aniseed has a great tendency to exist in a state of superfusion so that the oil may often be cooled down below its normal solidifying point, when it may be necessary to add a crystal of anethol to induce the oil to solidify, the temperature at the same time rising to what may be described as the correct solidifying point, which should not be below 13°.

A good deal of oil, which was possibly adulterated with a camphor oil fraction, is to be found from time to time on the London market. The author has examined a number of such samples recently.

On fractionating large samples of the oil in question, the first point to be noticed was the comparatively small amount distilling between 225° and 235°. In one case only 69 per cent was obtained, and in no case more than 75 per cent. The average for normal oils is 83 per cent. It was also noted that the first 10 per cent distilled had characters quite different from the corresponding fraction of pure oil. The following values are those of pure and suspected samples:—

Fraction.	Amount.	Pure Oil. M.Pt.	Suspected Oil. M.Pt.
	Per cent		
No. 1	10	9°	Not at 0°
No. 2	15	15°	12°
No. 3	20	19°	15°
No. 4	20	20°	17°
No. 5	20	20.5°	18°
Residue	15	14°	10°

The above are Umney's figures.

Fraction.	Amount.	Pure Oil. M.Pt.	Suspected Oil. M.Pt.
	Per cent		
No. 1	10	8°	- 3°
No. 2	25	18°	15°
No. 3	25	20°	17.5°
No. 4	25	20°	18°
No. 5 (Residue) . . .	15	15°	11°

The above are the author's figures.

It will be noticed that in every case the fraction of the suspected oil had a lower melting-point than the corresponding fraction of pure aniseed oil.

From the following figures it will be seen that the same fact is noticeable in regard to the refractive index, which were determined at 20° to 21°:—

Fraction.	Amount.	Pure Oil.	Suspected Oil.
	Per cent		
No. 1	10	1.5308	1.5110
No. 2	15	1.5470	1.5391
No. 3	20	1.5550	1.5463
No. 4	20	1.5575	1.5513
No. 5	20	1.5581	1.5538
Residue . . .	15	1.5540	1.5478

3. OLEUM ANTHEMIDIS.

This oil is distilled from the flowers of *Anthemis nobilis*.

The optical rotation varies from +1° to +3°. It consists principally of the esters of angelic and tiglic acids. Its use in medicine is extremely limited.

The British Pharmacopœia describes this oil as follows:—

“The oil distilled from Chamomile flowers.

“*Characters*.—Pale blue or greenish-blue when freshly distilled, but gradually becoming yellowish-brown. It should have the aromatic taste and odour of the flowers.

Specific gravity 0.905 to 0.915.”

Its refractive index is between 1.4440-1.4470

4. OLEUM CADINUM.

This oil contains a large proportion of Cadinine, one of the best-known sesquiterpenes. It is not an essential oil in the proper sense of the word, being obtained as mentioned below, by a process of destructive distillation.

The British Pharmacopœia describes this oil as follows:—

“An empyreumatic oily liquid obtained by the destructive distillation of woody portions of *Juniperus Oxycedrus*, Linn. [Moggridge, “Flora of Mentone,” tab. 65], and some other species.

“*Characters and Tests*.—A dark reddish-brown or nearly black, more or less viscid, oily liquid, with a not unpleasant empyreumatic odour and an aromatic, bitter and acrid taste. Specific gravity about 0.990. It is soluble in *ether* and *chloroform*; partially soluble in cold, almost wholly in hot *alcohol* (90 per cent). It is very slightly soluble in *water*. The filtered aqueous solution is almost colourless and possesses an acid reaction.”

5. OLEUM CAJAPUTI.

This oil is distilled from the leaves of various species of *Melaleuca*. The Pharmacopœia, however, restricts the oil to a given species.

The British Pharmacopœia describes this oil as follows:—

“The oil distilled from the leaves of *Melaleuca leucadendron*, Linn. (*Melaleuca cajaputi*, Roxb.) [“Bentl. and Trim. Med. Pl.” Vol. II, plate 108].

“*Characters and Tests*.—Bluish-green, with an agreeable penetrating camphoraceous odour, and an aromatic bitterish camphoraceous taste. Specific gravity from 0·922 to 0·930. It should become semi-solid on being stirred, when cold, with a third or half its volume of phosphoric acid of commerce of specific gravity 1·750 (presence of a due proportion of cineol).”

The specific gravity of the British Pharmacopœia is higher than that found in many samples of pure oil. It is well recognised that 0·917 is a permissible limit for genuine cajaput oil.

The oil is *nearly inactive* optically, the rotation usually varying from 0° to -2°. The refractive index varies from 1·4650 to 1·4680. Genuine oils contain from 55 per cent to 65 per cent of eucalyptol as determined by the phosphoric acid process, which is described under eucalyptus oil. The author has, in recent years, found no adulterants present in this oil except eucalyptus oil, which is detected by its odour, and alcohol, which can be estimated by shaking a known volume of the oil with ten times its weight of water. The oil is always of a pale green colour, but can be obtained white by redistillation. It owes its use in medicine entirely to the presence of eucalyptol.

6. OLEUM CARUI.

This oil is obtained by the distillation of the fruit of *Carum carui*. The British Pharmacopœia describes this oil as follows:—

“The oil distilled from Caraway fruit.

“*Characters*.—Colourless or pale yellow, with the characteristic odour of the fruit, and a spicy taste. Specific gravity 0·910 to 0·920.”

Caraway oil resembles Dill oil very closely in its composition, consisting practically entirely of carvone and limonene. Normal oils sometimes have a specific gravity slightly below that given in the British Pharmacopœia, but it is as well to adhere to the higher limit, as such low-gravity oils have usually been deprived of carvone. The oil has an optical rotation of +70° to +85°, and a refractive index 1·4870 to 1·4900. The usual adulteration of the oil consists either in the abstraction of carvone, or in the addition of oil from which carvone has been abstracted. The estimation of the carvone is therefore a matter of importance. This is best estimated by the process described under oil of cinnamon. The following process also yields excellent results:—

When 5 c.c. phenylhydrazine are added to 5 c.c. of caraway oil the mixture becomes warm owing to chemical combination taking place, and if the action be accelerated by placing the test tube in boil-

ing water for a few minutes a copious crystallization of carvone phenylhydrazone $C_{10}H_{14}:N.NH.C_6H_5$ appears, and on cooling the whole solidifies to a crystalline mass. After heating for one hour the reaction is complete, the excess of phenylhydrazine is removed by adding 5 c.c. glacial acetic acid whilst hot, shaking and diluting with 20 c.c. water. The contents of the test tube are then cooled and filtered through a paper disc by means of a pump, and the crystalline mass washed with water until of a pale yellow colour. By this process not only is the excess of phenylhydrazine removed in aqueous solution as acetate, but nearly all the oily terpene adherent to the crystals is washed away. On crystallizing from a definite volume of 95 per cent alcohol the carvone phenylhydrazone is obtained in long silky pale yellow needles, melting at $106^{\circ}C.$, but so difficult to dry without decomposition as to render the determination only approximate.

7. OLEUM CARYOPHYLLI.

This oil has already been described under the spice "Cloves".

8. OLEUM CINNAMONI.

This oil has been described under the spice "Cinnamon".

9. OLEUM COPAIBAE.

This oil will be found described under "Balsam of Copaiba".

10. OLEUM CORIANDRI.

This oil is distilled from the fruit of *Coriandrum sativum*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from Coriander fruit.

"*Characters and Tests.*—Colourless or pale yellow, having the odour and flavour of the fruit. Specific gravity 0.870 to 0.885. If 1 c.c. of the oil be mixed with 3 c.c. of alcohol (70 per cent), a clear solution results (absence of oil of turpentine and added terpenes)."

The optical rotation of this oil varies from $+7^{\circ}$ to $+15^{\circ}$. On fractional distillation from 45 per cent to 55 per cent should be obtained between 190° and 200° indicating a due proportion of linalol, which is the principal odorous constituent of the oil. The refractive index is about 1.4650 and the ester number varies from 4 to 23. The oil should be soluble in three times its volume in 70 per cent alcohol. The only adulterant now met with in this oil, is sweet orange oil, which interferes very greatly with the solubility of the oil and raises the optical rotation enormously.

11. OLEUM CUBEBAE.

This oil is the product of the distillation of the fruit of *Piper cubeba*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from cubebs.

Characters.—Colourless, pale-green, or greenish-yellow; with the

odour and camphoraceous taste of cubebs. Specific gravity 0.910 to 0.930."

The oil has an optical rotation of -30° to -40° and a refractive index of about 1.4950. The solubility of the oil in 90 per cent alcohol is very variable, some oils dissolving in one volume, others requiring 10 volumes to effect solution. The oil is a mixture of terpenes and sesquiterpenes, cadinine being the principal of the latter with a small amount of so-called cubeb-camphor. Samples are sometimes found adulterated with turpentine. A genuine oil on distillation yields the following fractions:—

Below 250° = 10 per cent; 250° to 260° = 25 per cent; 260° to 270° = 50 per cent; 270° to 280° = 5 per cent.

12. OLEUM EUCALYPTI.

This oil is distilled from the leaves of various species of *Eucalyptus*. Its reputation in medicine has been built up on a description under the name *Eucalyptus globulus*, although but little of the oil reaching this country under that name is really distilled from the *globulus* species.

In fact, to most patients the *globulus* oil is irritating and objectionable, and many oils distilled from other species are, in the author's opinion, much to be preferred to the *globulus* oil.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from the fresh leaves of *Eucalyptus globulus*, Labill. ["Bentl. and Trim. Med. Pl." Vol. II, plate 109] and other species of *eucalyptus*,

"*Characters and Tests*.—Colourless or pale yellow, having an aromatic camphoraceous odour, and a pungent taste, leaving a sensation of coldness in the mouth. Specific gravity 0.910 to 0.930. It should not rotate the plane of a ray of polarized light more than 10° in either direction in a tube 100 mm. long, and it should become semi-solid on being stirred, when cold, with a third or half its volume of phosphoric acid of commerce of specific gravity 1.750 (presence of a due proportion of cineol). If to 1 c.c. of the oil be added 2 c.c. of glacial acetic acid and 2 c.c. of a saturated aqueous solution of sodium nitrite, the mixture, when gently stirred, should not form a crystalline mass (exclusion of *eucalyptus* oils containing much phellandrene)."

Although there are many pure oils to be met with having figures well outside the limits given by the British Pharmacopœia there is a plentiful supply of oil up to the standards of that authority and it is wise that that standard should not be relaxed.

The British Pharmacopœia gives only a qualitative test for eucalyptol. A quantitative determination is therefore of considerable importance. No method, however, yields absolutely accurate results. It is therefore necessary in stating the eucalyptol value of a given oil, to describe the method by which the determination has been made. A convenient and approximately accurate method is as follows:—

To a known weight of oil from one to one and a half times its weight of phosphoric acid of specific gravity 1.75 should be added,

drop by drop, the oil being kept cold and continually stirred. The crystalline magma formed is pressed between filter paper, after as much as possible has drained off; and when the adherent terpenes and phosphoric acid have been removed as far as possible, the crystals are decomposed by hot water in a graduated tube. On cooling, the cineol is measured, and from its specific gravity (.930) the weight is easily calculated. The separated cineol should readily crystallize on cooling to -3° , otherwise it must be regarded as impure and the process repeated. Oils rich in cineol yield a correspondingly high fraction distilling between 170° and 190° .

The United States Pharmacopœia directs that the oil is to be diluted with petroleum ether before treatment with the phosphoric acid.

Messrs. Schimmel & Co. have recently recommended absorbing the eucalyptol by a 50 per cent solution of resorcinol in water, and reading the unabsorbed portion in the neck of an ordinary absorbing flask. Although this method gives approximately accurate results in some cases, so many other constituents of essential oils are absorbed by this reagent that the process cannot be entirely relied upon.

13. OLEUM JUNIPERI.

This oil is obtained by the distillation of the fully grown unripe fruit of *Juniperus communis*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from the full-grown unripe green fruit of *Juniperus communis*, Linn, ["Bentl. and Trim. Med. Pl." Vol. IV, plate 255]."

"*Characters and Tests*.—Colourless or pale-greenish yellow, with the characteristic odour of the fruit, and a warm, aromatic, bitterish taste. Specific gravity 0.865 to 0.890. The oil is soluble, with slight turbidity, in four times its own volume of a mixture of equal parts of absolute alcohol and alcohol (90 per cent)."

In regard to the above tests it should be noted that pure juniper oil rapidly loses its solubility by keeping, so that pure samples will frequently fail to comply with the solubility test of the Pharmacopœia.

Rectification also naturally alters the specific gravity, which depends chiefly on the relative proportions of terpene (specific gravity = 0.845), and sesquiterpene (specific gravity = .920). The limits .865 and .890 are certainly those which should be accepted for genuine good oils. The approximate proportions of pinene and cadinene may be judged by a fractional distillation, as pinene boils at 156° and cadinene at 274° . The results vary largely according to the fractionating apparatus used, but with a series of bulbs, from 25 per cent to 35 per cent is obtained between 155° and 160° , and at least 20 per cent over 200° , having a refractive index of over 1.4950 and a specific gravity over 0.904.

The oil is always lævorotatory, usually between -4° and -10° except in the case of Hungarian oil, which may have a rotation up to -19° . The refractive index varies from 1.4740 to 1.4880. After distilling off 90 per cent of the oil the 10 per cent residue should have a refractive index of not less than 1.5000 indicating a sufficient proportion of cadinine.

The only constituents of oil of juniper which have been ascertained with certainty are (1) the terpene, pinene $C_{10}H_{16}$; (2) the sesquiterpene, cadinene $C_{15}H_{24}$; (3) juniper camphor, a crystalline body probably belonging to the series of terpene alcohols; (4) an ester boiling at about 180° , probably the acetic ester of the above-mentioned alcohol. According to Schimmel, the chief, if not only, constituent of the stearoptene is a sesquiterpene alcohol melting at 165° to 166° .

14. OLEUM LAVANDULÆ.

This oil is distilled from the flowers of *Lavandula vera*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from the flowers of *Lavandula vera*, D. C. [Bentl. and Trim. Med. Pl." Vol. III, plate 199].

"*Characters and Test.*—Pale yellow or nearly colourless, with the fragrant odour of the flowers, and a pungent bitter taste. Specific gravity not below 0.885. It should dissolve in 3 times its volume of alcohol (70 per cent)."

There are two distinct varieties of genuine lavender oil; one is that distilled from the plants grown in certain districts in England, the other distilled principally from plants grown in France, and to some extent in Spain.

The chief difference between English and French oils of lavender lies in the fact that the former only contains about 7 to 10 per cent of esters calculated as linalyl acetate, whereas the latter contains up to 40 per cent and over. Messrs. Schimmel have actively endeavoured to establish this ester content as the basis of the valuation of the oil. They maintain the superiority of fine French oil over English oil, and go so far as to say that the latter cannot compete with the former. The author, in common with most others, holds the opposite opinion, and considers that no comparison can be made between the two oils on the basis of their ester content. This is much accentuated, if such were necessary, by the fact that linalyl acetate is not the odoriferous ingredient of oil of lavender. It is so much modified by the presence of other bodies, as to be regarded as only one of the odoriferous compounds in the oils. Pure linalyl acetate has a marked bergamot odour, and may be regarded as the characteristic ingredient of that oil. The fact that English oil fetches from 5 to 8 times as much as French oil speaks for itself. For a comparison of oils grown in the same locality, the ester comparison may, however, be of service. The oils produced in the south of Europe are finer according as the plants are growing at greater elevations, and according to Schimmel & Co. the very finest oils are produced from the higher valleys of the Savoy Alps, yielding 44 per cent of ester. The fine oils yielding 38 to 40 per cent of ester are usually obtained from the Alps Maritimes and the Basses Alpes, close to the Italian frontier. Less fine, but still excellent oils, with 28 to 32 per cent of ester, are obtained from the French departments of the Gard Drôme and Hérault.

Genuine lavender oil is a pale yellow oil of specific gravity 0.885 to 0.900, with an optical rotation of -3° to -10° . Rarely the specific

gravity falls to 0.883. The refractive index varies from 1.4622 to 1.4675. Coarse adulteration with such bodies as turpentine would reveal itself by the decrease in the solubility of the sample. Oil of spike lavender is used very commonly for the purpose of adulteration, and causes a reduction in the ester value, a rise in the specific gravity and usually a diminution in the optical rotation of the oil. Spike lavender oil being generally dextrorotatory, the most formidable adulterant with which the analyst has to cope to-day is a mixture of spike lavender oil and artificial esters, such as ethyl citrate, ethyl succinate, or ethyl oxalate. As these esters require considerably more alkali for saponification than does linalyl acetate, which is the principal constituent of French oil of lavender, a small quantity of any one of them appears in an ester determination, as indicating a considerably higher proportion of the natural ester.

The following is the best method for detecting adulteration with these artificial esters: ten grms. of the oil to be examined are saponified on the water bath for one hour with alcoholic potash, the contents of the flask then placed in a porcelain dish and the bulk of the alcohol evaporated. After this the liquid is washed in a separating funnel with about 100 c.c. water, the oil portions removed by extraction with ether, but the aqueous solution returned to the porcelain dish and the bulk evaporated on the water bath. When the alkaline solution has cooled down it is acidified with sulphuric acid, and the organic acids thus liberated absorbed by ether. In this manner a fine crystal residue remains behind. On recrystallization, however, from a small quantity of alcohol, white crystals are obtained, and the melting-point can be determined. In the event of the organic acid being insoluble in ether, it can be precipitated as a barium salt and examined. On fractional distillation the artificial esters will be found in the residues left after distilling off the more volatile portion, and will be found to have a very high specific gravity and low refractive index. A comparison with similar fractions of a normal oil will at once reveal the characteristic differences.

15. OLEUM LIMONIS.

This oil has been described under "flavouring essences".

16. OLEUM MENTHAE PIPERITÆ.

This oil is distilled from the flowering herb of *Mentha piperita*.

The British Pharmacopœia describes the oil as follows:—

"The oil distilled from fresh flowering peppermint, *Mentha piperita*, Sm. ["Bentl. and Trim. Med. Pl." Vol. III, plate 203].

"*Characters and Tests.*—Colourless, pale yellow, or greenish-yellow when recently distilled, but gradually becoming darker by age. It has the odour of the herb, and a strong penetrating aromatic taste, followed by a sensation of coldness in the mouth. Specific gravity 0.900 to 0.920. It should dissolve in four times its volume of alcohol (70 per cent). If a portion of the oil be cooled to 70° F. (–8.3° C.)

and a few crystals of menthol be added, a considerable separation of menthol should take place."

So far as English commerce is concerned the only true oils of peppermint which come under consideration are the English and American distillates. It is true that a large business is done in Japanese peppermint oil but this is distilled from a different species—*Mentha arvensis*. The figures given here in regard to the oil refer to these two species only. Few plants alter more largely in the character of their essential oil according to the districts in which they are cultivated than does peppermint, so that French, Italian, and Spanish oils have characters quite different from those here discussed, but as they do not enter into English commerce to any extent they need not be further considered. The principal constituent of peppermint oil is menthol, principally in the free condition and to a smaller extent as esters, together with a certain amount of menthone. Numerous other bodies exist in this oil, for an account of which the author's work "The Chemistry of Essential Oils" should be consulted. True peppermint oil has a specific gravity of 0.900 to 0.920, rarely up to 0.925, and an optical rotation of -22° to -33° , and refractive index up to about 1.4650. It is soluble in from 3 volumes to 4 volumes of 70 per cent alcohol but in the case of certain American oils, the solubility is not complete. American and English oils contain from 50 per cent to 65 per cent of menthol. The Japanese oil has a specific gravity of 0.895 to 0.905 and an optical rotation of -25° to -43° . The menthol of commerce is almost entirely derived from Japanese peppermint oil. The normal Japanese oil contains over 70 per cent of menthol, and after the abstraction of a portion of this, the residual oil is sold on this market as Japanese dementholized peppermint oil and contains about 40 per cent of menthol. The table on page 616, due to Power and Kleber, shows the characters of a number of typical peppermint oils:—

The most important determination apart from the physical characters for this oil, is the estimation of the amount of menthol. The following are the details of the necessary process:—

About 10 grms. (accurately weighed) of the oil together with 20 c.c. of an alcoholic normal solution of sodium hydroxide, are either heated to boiling for half an hour in a flask provided with a reflux condenser, or the mixture, contained in a strong, securely-stoppered glass bottle, is heated for an hour in a bath of boiling water, and subsequently the uncombined alkali titrated with normal sulphuric acid with the use of phenol-phthalein as an indicator. From this the combined menthol is calculated as menthyl acetate.

The saponified oil is then repeatedly well washed with water and finally boiled for 2 hours with an equal volume of acetic anhydride and 2 grms. anhydrous sodium acetate in a flask provided with a suitable condensing tube, ground at one end so as accurately to fit the neck of the flask. The product, after cooling, is washed with water, then with a dilute solution of sodium carbonate, dried in contact with calcium chloride, and filtered. From 3 grms. to 4 grms. of the resulting oil are then saponified as above, now using 25 c.c. of

alcoholic normal solution of sodium hydroxide, and the uncombined alkali determined by titration.

As each c.c. of normal alkali required for saponification corresponds to 0.156 grm. menthol, and as this yields 0.198 grm. menthyl acetate, it is necessary, in order to calculate the found amount of menthol with reference to its percentage in the non-acetylied oil (free from ester), to subtract from the amount of oil used for saponification 0.042 grm. (the difference between 0.156 grm. and 0.198 grm.) for each c.c. of normal alkali consumed. If, for example, s grms. of acetylied oil had required for saponification a c.c. of normal alkali, the total percentage P of menthol, free and in the state of ester, may be calculated by the following formula:—

$$P = \frac{a \times 15.6}{s - (a \times 0.042)}$$

This, indeed, does not indicate with absolute exactness the percentage of menthol contained in the original oil, for it is assumed in this calculation that all the menthol which is present as ester is combined with acetic acid, whereas as a matter of fact it is partly in combination with iso-valerianic acid, etc. But the error so introduced is so small that it may be left out of account.

As menthone may readily be converted into menthol by reduction, the above-described method may be also employed for the determination of the amount of menthone in the oil, in the following manner. The oil is saponified, and in a portion of the product, previously deprived of alcohol, the percentage of menthol is determined. Another portion is diluted with twice its volume of alcohol, and treated at the boiling temperature of the liquid with metallic sodium. Of the oil which separates by the subsequent addition of water, a weighed quantity is used for another estimation of menthol. The increase corresponds to the amount of menthone present.

The above formula, which gives the total percentage of menthol, is not quite accurate, as it is referred to the ester-free (saponified) oil. The correction necessary to be introduced is not of great importance, as the quantity of menthyl esters is not nearly so great as that of free menthol, but to be perfectly correct it must be remembered that to calculate the ester-free oil to the original peppermint oil, the latter has lost weight as compared with the former to the extent of .75 per cent for each 1 per cent of KOH required for the preliminary saponification of the esters, assuming that these are all present as menthyl acetate. Thus if an oil gives an ester content of 10.6 per cent, equivalent to 3 per cent of KOH, or 8.4 per cent of menthol, and a total menthol content as calculated from the above formula of 60 per cent, it is necessary to multiply this by the factor $\frac{100}{102.3}$ to obtain the total men-

thol content in the original oil, i.e. 58.7. Hence the free menthol will be 50.3 per cent and the combined menthol 8.4 per cent.

Peppermint oil is frequently adulterated. The American oil is sometimes enriched by the addition of menthol; or it is adulterated with camphor oil, petroleum oil, cedar wood oil, and African copaiba

oil. In one case Bennet has observed the use of glyceryl triacetate. Camphor oil, petroleum oil, cedar wood oil and copaiba oil impair the solubility so seriously as to at once be indicated. Glyceryl triacetate is usually soluble in spirit and is therefore not indicated by the solubility test. The fractional distillation of the oil is absolutely essential in considering adulterants of this type. The pure oil will give figures not differing materially from the following, which were obtained on a normal sample:—

Quantity.	Specific Gravity.	Opt. Rotation.	Refractive Index.
Per cent			
12½	0·898	+ 10	1·4660
"	0·903	- 14	1·4635
"	0·907	- 16	1·4645
"	0·910	- 20	1·4640
"	0·912	- 23	1·4615
"	0·912	- 23	1·4615
"	0·915	- 24	1·4630
"	0·962	—	1·4790

If cedar wood or copaiba oils are present the higher boiling fractions will be found to have refractive indices up to 1·490 or even higher, and to consist of hydrocarbons quite insoluble in 70 per cent or 80 per cent alcohol. With cedar wood oil the rotations of the higher boiling fractions may reach - 45° and in the case of African copaiba will be much lower than normal. In the case of glyceryl triacetate, the residues after distilling off the more volatile portion of the oil will be found to have a high specific gravity even up to 1·14, and a low refractive index down to 1·445; such residues will also be found to be freely soluble in 70 per cent or 80 per cent alcohol.

17. OLEUM MENTHÆ VIRIDIS.

The true spearmint oil is obtained from the green herb *Mentha viridis*, but a good deal of the oil of commerce is obtained from *Mentha crispa*. The two oils however are practically identical.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from fresh flowering spearmint, *Mentha viridis*, Linn. ["Bentl. and Trim. Med. Pl." Vol. III, plate 202].

"*Characters and Tests*.—Colourless, pale-yellow, or greenish-yellow when recently distilled, but becoming darker by age. It has the odour and taste of the herb. Specific gravity 0·920 to 0·940. The oil forms a clear solution with its own volume of a mixture of equal parts of absolute alcohol and alcohol (90 per cent)."

The specific gravity of the oil may reach considerably higher limits than those of the B. P., a pure oil with a specific gravity of over ·970 having been observed by Schimmel & Co. The optical rotation of the oil usually varies between - 40° and - 50°. The oil should dissolve in 1 volume of 90 per cent alcohol.

When estimated as described under cinnamon oil this oil should show a carvone content of between 35 per cent and 45 per cent, and on fractional distillation about that quantity should be obtained between 200° and 226°. The oil is not much applied in medicine.

18. OLEUM MYRISTICÆ.

This oil has already been described under the spice "Nutmeg".

19. OLEUM PIMENTÆ.

This oil has already been described under the spice "Allspice".

20. OLEUM PINI.

There are numerous varieties of essential oils distilled from pine leaves, the British Pharmacopœia only recognizing that distilled from the leaves of *Pinus pumilio*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from the fresh leaves of *Pinus pumilio*, Haenke [Lambe. Gen. Pin. I. plate 2].

"*Characters and Tests.*—Colourless or nearly so, with a pleasant aromatic odour and pungent taste. Specific gravity 0·865 to 0·870. It should rotate the plane of a ray of polarized light from 5° to 10° to the left at 60° F. (15·5° C.) in a tube 100 mm. long. Not more than 10 per cent should distil below 329° F. (165° C.)."

A genuine oil may have a specific gravity up to ·875, but according to Umney should give only a minute distillate below 165°, a typical sample examined by him yielding only 2 per cent. About 60 per cent should distil between 165° and 180°.

Many samples of pine needle oil correspond with the description and tests of the British Pharmacopœia, but are in reality distilled from other species of pine.

21. OLEUM ROSÆ.

This oil is distilled from the flowers of *Rosa damascena* which is the only official variety recognized by the British Pharmacopœia although French oil of rose is distilled from *Rosa centifolia*.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled from the fresh flowers of *Rosa damascena*, Linn. [Redouté, "Les roses," plate 109].

"*Characters and Tests.*—A pale yellow crystalline semi-solid, with the strong fragrant odour of rose and a sweet taste. Specific gravity 0·856 to 0·860 at 86° F. (30° C.). The congealing and melting-points vary according to the proportions of crystalline matter, but should lie between 67° and 72° F. (19·4° and 22·2° C.)."

The Pharmacopœial description of this oil practically restricts it to the Bulgarian product. Few essential oils are so grossly adulterated as is this, but as its use is exceedingly restricted in medicine, it only being used to perfume a few preparations, there is no justification for devoting much space to it in a work devoted to food and drugs.

When the otto has to be examined from a perfumer's point of view reference should be made to the "Chemistry of Essential Oils," by the author, second edition, pp. 396-409.

The following, however, are to be regarded as the limits for the figures yielded on analysis by pure Bulgarian otto of rose :—

Specific gravity at 30°	0.850 to 0.861
Optical rotation	-2 „ -5°
Refractive index at 25°	1.4610 „ 1.4650
Melting-point	19° „ 23°

The total alcohols calculated as geraniol, when estimated in the method similar to that described for menthol and oil of peppermint, should not exceed 75 per cent or rarely 76 per cent. A small quantity of alcohol is frequently added as an adulterant. This may be detected by shaking the otto with warm water and testing the water separated by the usual iodoform reaction. Otto so washed with water will if alcohol be present show a rise in its refractive index. A pure otto in these circumstances will not show an increase in its refractive index of more than 0.002.

Further, if instead of using acetic anhydride to convert the alcohols into esters as in the determination of the total alcohols, formic acid be used, the citronellol, which is one of the constituents of the alcohol of this oil, will alone be esterified, so that separation of the geraniol and citronellol is thus practicable. In genuine otto of rose the approximate amount of citronellol in the total alcohols will be about 35 per cent. A much lower figure than this indicates the addition of geraniol prepared from other sources.

22. OLEUM ROSMARINI.

This oil is distilled from the flowering tops of *Rosmarinus officinalis*.

The British Pharmacopœia describes this oil as follows :—

"The oil distilled from the flowering tops of *Rosmarinus officinalis*. Linn. ["Bentl. and Trim. Med. Pl." Vol. III, plate 207].

"*Characters and Tests*.—Colourless or pale yellow, with the odour of rosemary, and a warm camphoraceous taste. Specific gravity 0.900 to 0.915. It should dissolve in twice its volume of alcohol (90 per cent), and should not rotate the plane of a polarized ray of light more than 10° to the right in a tube 100 mm. long (absence of oil of turpentine)."

In regard to the British Pharmacopœia figures given above, it may be remarked that many samples of pure rosemary oil, especially those distilled in Spain, are laevorotatory up to -9°.

Apart from the determination of the physical characters of this oil the only estimation that is usually necessary is that of the boneol, which is determined in a manner similar to menthol in oil of peppermint. This will usually vary from about 12 per cent in low-grade samples to 20 per cent in the best samples.

23. OLEUM SANTALI.

This oil is distilled from the wood of *Santalum album*.

The British Pharmacopœia describes this oil as follows :—

“The oil distilled from the wood of *Santalum album*, Linn. [“Bentl. and Trim. Med. Pl.” Vol. IV, plate 252].

“*Characters and Tests*.—Somewhat viscid in consistence, pale yellow in colour, having a strongly aromatic odour and a pungent and spicy taste. Specific gravity 0·975 to 0·980. It forms a clear solution with six times its volume of alcohol (70 per cent) (absence of cedar wood oil). It rotates the plane of a ray of polarized light to the left, through an angle not less than 16° and not more than 20°, in a tube 100 mm. long (absence of other varieties of sandal wood oil).”

This oil consists essentially of from 90 per cent to 95 per cent of alcohols, which although a mixture of several bodies, are usually known as santalol, to which the formula $C_{15}H_{26}O$ is usually assigned. The chemistry of this oil is fully discussed in the work above quoted, by the author, pp. 244 to 260. The figures of the Pharmacopœia are not sufficient to determine the purity or otherwise of this oil. A genuine oil has a specific gravity varying between 0·973 and 0·985. The optical rotation occasionally falls below -16°, but as a rule when this is the case it is due to defective distillation of the wood. The oil has a refractive index of 1·505 to 1·510. It contains a minute quantity of free acid and requires 0·7 gm. to 1·5 grms. of potassium hydroxide to saponify the esters present. The alcohols calculated to the above given formula, when estimated by a process similar to that described for menthol in oil of peppermint, should never fall below 90 per cent. A pure oil rarely shows below 92 per cent. Most adulterants cause the oil to be considerably less soluble than the pure oil should be, and also reduce the amount of total alcohols, which is usually known as the santalol value. Fractional distillation of the oil may occasionally be necessary. When this is the case, the results should agree with the following, which were obtained on a normal pure sample of sandal wood oil :—

Fraction.	Specific Gravity.	Opt. Rotation.	Refract. Ind.
	Per cent		
1	0·970	-19° 30'	1·5055
2	·970	-17° 20'	1·5060
3	·927	-16°	1·5060
4	·974	-16°	1·5065
5	·977	-15° 30'	1·5068
6	·978	-15°	1·5068
7	·980	-16° 40'	1·5079
8	·980	-18°	1·5080
9	·984	-21°	1·5084

The acetylated oil should have a specific gravity 0·986 to 0·989, an optical rotation of -13° 30' to -18° and a refractive index 1·4899 to 1·4920 at 20°.

24. OLEUM SINAPIS VOLATILE.

This oil has been described under the condiment "Mustard".

25. OLEUM TEREBINTHINÆ.

This oil is obtained by the distillation of the oleo-resin obtained from *Pinus sylvestris* and other species of pine.

The British Pharmacopœia describes this oil as follows:—

"The oil distilled, usually by the aid of steam, from the oleo-resin (turpentine) obtained from *Pinus sylvestris*, Linn. ["Bent. and Trim. Med. Pl." Vol. IV, plate 275], and other species of *Pinus*; rectified if necessary.

"*Characters and Tests.*—Limpid, colourless, with a strong peculiar odour, which varies in the different kinds of oil, and a pungent and somewhat bitter taste. It is soluble in its own volume of glacial acetic acid. It commences to boil at about 320° F. (160° C.), and almost entirely distils below 356° F. (180° C.), little or no residue remaining."

The following are the best-known varieties of oil of turpentine:—

American Turpentine.—This is chiefly obtained from *Pinus Australis*, but also to a certain extent from *Pinus taeda*, the loblolly pine. It is a colourless limpid liquid of specific gravity ·855 to ·870. It is almost invariably dextrorotatory, to the extent of about + 3° to + 15°, but is rarely slightly lævorotatory. It commences to boil at 156° to 157°, and in good samples 88 per cent to 99 per cent will distil below 165°. Its chief constituent is pinene $C_{10}H_{16}$ and a little dipentene is also present.

French Oil of Turpentine.—This variety is chiefly obtained from the oleo-resin of *Pinus pinaster*. Here again the chief constituent is the terpene pinene, and the great difference between this and American turpentine lies in the fact that the former is lævorotatory, about - 18° to - 40°.

German Oil of Turpentine is chiefly the product of *Pinus sylvestris*, but *Pinus abies*, *Pinus vulgaris* and *Pinus picea* also contribute to it. Its specific gravity is ·860 to ·870, and it is dextrorotatory, about + 15 to + 20°. It contains pinene and sylvestrene.

Russian and Swedish Oil of Turpentine.—This variety is almost entirely obtained from *Pinus sylvestris* and *Pinus ledebourdii*. In general properties it resembles German oil, but it is rather more variable in specific gravity, etc. According to Tilden, it contains as much as 60 per cent to 70 per cent of sylvestrene. Its specific gravity is usually about ·870 to ·875, and its boiling-point about 170°. It is dextrorotatory to the extent of + 20°. Of all the commercial turpentines it is of the least technical importance. It often possesses a disagreeable empyreumatic odour, due to the presence of the products of destructive distillation of the pine-wood.

Other less important turpentines are Hungarian (from *Pinus pumilio*); Austrian (from *Pinus laricio*); Carpathian (from *Pinus cembra*), and Finnish (similar to German). In addition, Venetian turpentine and Canada balsam yield oils. These latter, however, have practically no commercial interest.

The terebene of pharmacy consists of optically inactive terpenes, the result of the action of sulphuric acid on turpentine, which causes a certain amount of isomerization, and also changes the active terpenes into their inactive variety. For the manufacture of this, it is preferable to employ rectified oil of turpentine. Indeed, for pharmaceutical purposes in general, it is usual to employ turpentine purified by redistillation.

Turpentine is sometimes adulterated with petroleum and with rosin spirit, and, rarely, with volatile portions of shale oil and coal tar. It is itself used very largely to adulterate other essential oils, both on account of its price and because it so closely resembles many other oils in chemical constitution.

The accompanying table (p. 624) is given by Allen (*Commercial Organic Analysis*), embracing certain properties of these bodies.

The chief points of importance to be noted in the examination of the oil are the specific gravity, boiling-point and temperature of distillation, optical activity, and flashing-point.

Good commercial turpentine has a specific gravity of $\cdot 858$ to $\cdot 870$, only occasionally passing these limits slightly. Russian oil has a higher gravity—often reaching $\cdot 875$. The optical activity, as stated above, varies with the source, and this factor is only of value when studied in conjunction with the other features of the oil. The boiling-point is usually 155° to 156° , and a considerable portion distils at below 160° . In the best class of oils at least 85 per cent distils below 165° , often several degrees below this temperature. Russian oil, on the other hand, distils chiefly between 170° and 180° . When adulterated, the temperature of distillation rises gradually, and no large fractions are obtained at any definite temperature when the adulteration is at all excessive. The presence of ordinary petroleum spirit lowers the flash point of turpentine. When pure, it flashes at 92° to 95° F. when tested in Abel's flash-point apparatus. With only 1 per cent of ordinary petroleum spirit this temperature is reduced by 10° .

According to Armstrong, a good indication of the presence of the usual adulterants is obtained by distillation with steam. A current of steam is allowed to pass into a definite volume of the turpentine contained in a flask attached to a condenser. Unless it has been allowed free access to the air for some time, the genuine oil leaves only traces of non-volatile matter, but old samples may leave up to 2 per cent.

Usually, however, the presence of more than $\cdot 5$ per cent after steam distillation indicates the presence of unvolatilized petroleum oil. This is easily recognised by its low specific gravity and its fluorescence when dissolved in ether. If the residue consists of resin oil, it will form a bulky soap when rubbed with slaked lime. The specific gravity of the fractions coming over with the steam will largely assist in determining the presence of volatile adulterants.

For the approximate estimation of the amount of petroleum naphtha in adulterated turpentine, Armstrong recommends the following process: 500 c.c. of the sample are placed in a separator and treated with about 150 c.c. of sulphuric acid (two volumes of acid to one of water). The mixture is cautiously agitated, and if much rise

	Turpentine Oil.	Rosin Spirit.	Petroleum Naphtha.	Shale Naphtha.	Coal-tar Solvent Naphtha.
1. Optical activity . . .	active	usually none	none	none	none
2. Specific gravity860 to .872	.856 to .880	.700 to .740	.700 to .750	.860 to .875
3. Temperature of distillation .	156° to 180°	gradual rise	gradual rise	gradual rise	gradual rise
4. Action in the cold on coal-tar pitch	readily dissolves	readily dissolves	very slight action	very slight action	readily dissolves
5. Behaviour with absolute phenol at 20° . . .	homogeneous mixture	homogeneous mixture	no apparent solution	homogeneous mixture, crystallizes on cooling	homogeneous mixture
6. Behaviour on agitating 3 vols. with 1 vol. castor oil . .	homogeneous mixture	homogeneous mixture	two layers of nearly equal volume	like petroleum naphtha	—
7. Bromine absorption (dry) .	203 to 236	184 to 203	10 to 20	60 to 80	—
8. Behaviour with H_2SO_4 . .	almost entirely polymerized	polymerized	very little action	considerable action	moderate action

of temperature is observed, the separator must be placed in cold water for a short time. The turpentine is gradually converted into a viscid oil, and when this has taken place, and no more heat is developed on repeated agitation, the acid is tapped off. The oily layer is then transferred to a flask and subjected to steam distillation. When all that is volatile with steam has passed over, the oily portion of the distillate is separated from the aqueous layer, and heated with half its volume of sulphuric acid previously diluted with one-fourth of its measure of water. The mixture is well agitated, the acid liquid separated, and the oily layer again distilled with steam. When genuine turpentine is operated upon, the volatile portion of this second treatment consists merely of cymene and a small quantity of paraffinoid hydrocarbons. It never exceeds 4 to 5 per cent of the volume of the original sample, and with care is as low as 3 per cent. If the volume notably exceeds 5 per cent, it is advisable as a precaution to repeat the treatment with the (4 to 1) acid. When treated in this manner, petroleum naphtha is not appreciably affected, hence the proportion may be fairly estimated by making an allowance of 4 to 5 per cent from the volume of volatile oil which has survived the repeated treatment with sulphuric acid. A further purification may be effected by violently agitating the surviving oil with several times its volume of concentrated sulphuric acid heated to 50° or 60°. This treatment can be repeated if necessary, after which the residual hydrocarbon is separated, steam-distilled, and again measured, when the surviving oil from pure turpentine oil will not exceed from one half to one per cent of the original sample. Any excess over this will be the minimum quantity of petroleum naphtha present. Shale naphtha cannot be at all estimated in this way. The behaviour of the oil on distillation is the best indication of the presence of rosin spirit, as the temperature rises gradually, and no considerable fraction is obtained at 158° to 160° if much rosin spirit be present.

CHAPTER XI.

FATTY OILS, WAXES AND SOAPS OF THE BRITISH PHARMACOPŒIA.

THE examination of the fixed oils is well understood, and the only difficulty in the analysis of these bodies is the interpretation of results, which is rendered all the more difficult on account of the fact that the figures obtained for various oils frequently overlap to a considerable extent. Apart from the determination of the usual physical constants of an oil, the following are special processes which are usually necessary to adopt in their examination:—

Saponification.—The saponification value, or Koettstorfer value, of a fat or wax is the number of mgs. of KOH requisite for the complete saponification of a given sample. As a rule a fatty oil contains some free oleic or similar acid, and a certain amount of alkali is necessary to neutralize this, but generally speaking one understands the saponification value to include the amount of KOH necessary for the neutralization of the free acids as well as that for the hydrolysis of the esters. If these values are expressed separately they become the acid and ester values respectively, being the number of mgs. of KOH necessary to neutralize the acids, and decompose the esters respectively.

For the determination of these values the following process should be used. About 2 grms. to 4 grms. of the oil or wax accurately weighed is warmed with about 10 c.c. of alcohol, and well agitated with it. A few drops of phenol-phthalein are added, and alcoholic potash of about semi-normal strength run in until the pink colour is permanent. The amount of alkali used is noted and a further 25 c.c. of the alcoholic potash solution run in. The liquid is now boiled briskly for thirty minutes under a reflux condenser. After cooling it is diluted with 50 c.c. of water, and semi-normal hydrochloric acid run in until the pink colour is discharged. A blank experiment is conducted at the same time, using the same reagents but omitting the oil. This will give the exact value of the potash solution. From these results, the amounts of potash used for the neutralization of the free acids and for the hydrolysis are given, and expressed in mgs. per gram of the sample give the acid and ester—or added together—the saponification value of the fat.

Characters of the Fatty Acids.—In order to determine the amount and character of the fatty acids, 10 grms.—or 5 grms. if it is not necessary to make a very full examination—of the sample is saponified

with 125 c.c. of a semi-normal alcoholic potash solution. The bulk of the alcohol is evaporated, water added, the whole warmed to ensure complete solution, and the fatty acids liberated by HCl, and allowed to rise to the surface. The lower layer is run off, the fatty acids washed twice in a separator with hot water, and finally filtered, dried and weighed. The iodine value, neutralization value and melting-point can then be determined on these in the usual manner. The neutralization value is the number of mgs. of KOH necessary to neutralize 1 gm. of the fatty acids. This is determined by dissolving about 1 gm. in 10 c.c. of alcohol, and titrating with semi-normal alkali, using phenol-phthalein as indicator. The mean molecular weight can be calculated from this value, as the molecular weights of the free acids, and that of potassium hydroxide are in direct proportion to the amounts of the two which neutralize each other. The melting-point is determined in the usual manner in a capillary tube, the acids being first cooled on ice and allowed to stand thereon for an hour before the determination is made. The iodine value is determined as in the case of the oil itself, except that the fatty acids do not require the addition of any chloroform, as they are soluble in the alcoholic solution of iodine.

Unsaponifiable Matter.—The unsaponifiable matter may be determined on the quantity of oil used for the determination of the saponification value. The saponification liquor is evaporated on a water bath, with the addition of water, until the alcohol is driven off. The cold aqueous solution is then transferred to a separator and shaken with its own volume of ether. This is allowed to separate, and then the aqueous layer is run off. This is repeated twice, and the mixed ethereal solutions are washed in a separator with a little distilled water. The water is run off, and the ether filtered if necessary. The ether is evaporated and the unsaponifiable matter dried and weighed.

Determination of the Hehner Value.—This value is generally understood as the percentage of insoluble fatty acids present in an oil.

Three to 5 grms. of the oil are saponified with alcoholic potash in the usual manner, and the alcohol removed by evaporation. The soap is dissolved in water, and decomposed by excess of sulphuric acid. The liquid is then warmed until the free fatty acids float on the surface of the liquid as an oily layer. A weighed quantity—about 2 grms. to 3 grms. of dry hydrocarbon wax—is now added, and when melted the whole is well stirred and allowed to cool. The solid cake of fatty acids and neutral wax is then freed from the aqueous liquid by piercing the cake by a glass rod, and pouring off the liquid. Hot water is then added, and the cake remelted, stirred with the water, and allowed to set again. This washing is repeated until the wash water is free from acid, when the cake is removed, adherent water removed by absorbent paper, and the cake transferred to a porcelain dish, dried at 105°, and weighed. The weight, minus the weight of wax added, gives the fatty acids insoluble in water. Most fats contain about 95 per cent of such acids, but the following deviate to a considerable extent from this figure:—

	Per cent.
Maize oil	88.5 to 95
Shark liver	88 „ 93
Many fish oils of uncertain origin	75 „ 85
Cocoa-nut oil	88 „ 90
Japan wax	89 „ 92
Butter	86 „ 90

The Iodine Value.—The iodine value is an expression of the amount of iodine (as a percentage of the fat used) which will combine with the fat under definite conditions. This value denotes the amount so combined, when the conditions laid down by Hubl are observed. An alternative process is that of Wijs, but as Hubl's process is still generally employed, the iodine value without qualification is here intended to mean the value as determined by Hubl's process. The Wijs values are qualified by the use of the name of the chemist responsible for the process.

Hubl's process is carried out as follows:—

Solutions Necessary.—*The Iodine Solution* is prepared by dissolving 25 grms. of pure iodine in 500 c.c. of 95 per cent alcohol: and 30 grms. of mercuric chloride in a separate portion of 500 c.c. of alcohol of the same strength. The two solutions are then mixed, and allowed to stand for at least three days before use (twenty-four hours is generally said to be sufficient, but the author finds a gradual diminution in iodine value of the mixed solution goes on for quite two days, and it is safer to allow the solution to stand for three days). A small diminution goes on for a long time after the three days, but this is not important, as standard blank experiments are always carried out when an iodine determination is being made.

The Thiosulphate Solution.—About 25 grms. of pure sodium thiosulphate are dissolved in 1000 c.c. of water. This is standardized at least once a week by weighing out about 0.25 gm. of pure re-sublimed iodine, dissolving in 5 c.c. of water containing 3 grms. of potassium iodide, and diluting to 25 c.c. The thiosulphate is run in from a burette, until the yellow colour is nearly discharged, when a little starch solution is added. The thiosulphate is then carefully run in until the blue colour is just discharged. The number of c.c. used divided into the number of mgs. of iodine used will give the iodine value of the thiosulphate solution.

From 0.1 to 0.2 of the sample—according to its probable iodine value—is weighed into a stoppered bottle holding about 250 c.c., and 10 c.c. of pure chloroform is added; 25 c.c. of the iodine solution are then added, and the whole mixed by rotating the bottle. The bottle is then allowed to stand, concealed from the light, for about eighteen hours. At the same time a blank experiment is conducted, the bottle containing the reagents in the same quantity, but none of the sample.

At the end of eighteen hours, the contents of the bottles are titrated. The bottle containing the sample should be of a deep brown colour, so that not more than half the iodine used has been consumed. If on titration it should be found that substantially less than half the original quantity of iodine remains, then the recorded iodine value is probably too low, and the experiment should be repeated.

To each of the bottles 10 c.c. of a 10 per cent of potassium iodide solution in water is added, and after gentle agitation to mix the liquids, 50 c.c. to 100 c.c. of water are added. Thiosulphate solution is then run in, until the yellow colour of the aqueous solution and the red colour of the chloroform are nearly discharged. The liquid is then well agitated in order to cause the remaining iodine to pass entirely into the aqueous solution, and a little starch solution added. Thiosulphate solution is then run in until the colour is discharged, and does not immediately return on agitation of the contents of the bottle. The number of c.c. required for the blank experiment, minus the number required for the sample, gives the iodine in terms of thiosulphate that has combined with the oil. Since the iodine value of the thiosulphate solution is known, the actual iodine absorbed is easily calculated, and from this the iodine value (per cent of iodine absorbed) is deduced. In the case of fatty acids, the chloroform may be omitted.

The Wijs process depends on the use of a solution of iodine trichloride in glacial acetic acid. The advantages of this solution are that it does not alter materially in strength even after keeping for a long time, and that the process can be completed in under an hour. The absorption should be allowed to go on for thirty minutes, or in the case of drying oils with an iodine value of over 100, for one hour.

The iodine solution is prepared by dissolving 9.4 grms. of iodine trichloride and 7.2 grms. of iodine in glacial acetic acid on the water bath, each being dissolved in separate portions. The solutions are then mixed and made up to 1000 c.c. with the acid. The chloroform used in Hubl's process must be replaced by carbon tetrachloride, as chloroform often contains traces of alcohol, which interfere with the reaction.

Otherwise the test is carried out as described above, except that the absorption should only go on for thirty to sixty minutes. The results are practically identical with those obtained by Hubl's process (but this is not the case with resins, which appear to behave in an erratic fashion with the Wijs solution).

The Bromine Thermal Value.—This test, due to Hehner and Mitchell, depends on the rise in temperature of a given quantity of the oil when mixed with a given quantity of bromine under definite conditions. This value is in close relation to the iodine value, and the latter may be approximately calculated from the bromine value. A convenient method of applying this test is as follows:—

Five grms. of the oil are dissolved in 25 c.c. of chloroform, and 5 c.c. of this solution are placed in a small Dewar's vacuum tube, taking care that the liquid does not flow down the side of the tube. The temperature of the liquid is taken with a thermometer graduated in one-fifth degrees. A solution of 1 volume of bromine in 4 volumes of chloroform is prepared, and brought to the same temperature as the oil solution, poured into the vacuum tube, the whole gently stirred with the thermometer, and the rise in temperature noted.

Hehner and Mitchell in their original test use 1 c.c. of pure bromine and 1 gm. of oil in 10 c.c. of chloroform. Under these circumstances the bromine thermal value $\times 5.5$ gives a very close approximation to

the iodine value. The following values were obtained by Hehner and Mitchell :—

Lard	10.6°
Butter	6.6°
Olive oil	15°
Corn o-l	21.5°
Cottonseed oil	19.4°

The Detection and Separation of Cholesterol and Phytosterol.—

These similar, and probably isomeric, alcohols are found, the former in numerous animal oils, the latter in most vegetable oils. The presence of phytosterol is considered conclusive evidence of the presence of a vegetable fat. The best method for the separation of these bodies is that of Börner ("Zeit. Unter. Nahr. Genuss." 1898, 1, 31). He saponifies 100 grms. of the fat with 200 c.c. of 20 per cent alcoholic potash, dilutes the liquid with 400 c.c. of water and shakes the whole, when cold, with 500 c.c. of ether. This is separated, and the soap solution extracted three times more with 250 c.c. of ether. The ether is distilled off and any traces of alcohol present removed by heating on the water bath. The residue is again boiled with a little alcoholic potash in order to be certain that all fat is saponified, and the liquid diluted with water and again thoroughly extracted with ether. The ether is washed with water and filtered and evaporated, leaving the crude cholesterol and phytosterol. In the case of animal fats, the residue is chiefly cholesterol, whilst with vegetable fats it is mostly phytosterol. It is dissolved in about 10 c.c. to 15 c.c. of absolute alcohol with the aid of heat, and the liquid allowed to deposit crystals in a shallow dish. In the case of cholesterol alone, crystallization commences at the margin and gradually extends towards the centre of the liquid on the surface. This crop, which soon separates, is separated and dried on blotting paper. With phytosterol no surface film is formed, but needles are separated from the margin inwards, under the surface of the liquid. These crystals are best separated by filtration. The crystals may be washed with a very small amount of absolute alcohol and then examined microscopically. The general appearance of (1) pure cholesterol, (2) pure phytosterol, (3) mixtures of both, are shown by the following diagrams on opposite page.

The crude alcohols may also be dissolved in the smallest possible amount of absolute alcohol and allowed to crystallize, and after microscopic examination, the crystals obtained, together with the residue left after evaporating the alcohol, are boiled in a small dish, covered with a watch-glass, with 2 c.c. to 3 c.c. of acetic anhydride. The excess of acetic anhydride is driven off on the water bath, and the residue dissolved in a little hot absolute alcohol, so that crystallization shall not take place directly the alcohol cools. Allow the crystals to separate slowly, and when about half the alcohol has spontaneously evaporated, remove the crystals with a spatula, and wash them in a small filter with 3 c.c. of 95 per cent alcohol. Redissolve in 5 c.c. to 10 c.c. of absolute alcohol and again allow to crystallize. The crystallization should be repeated five to six times, the melting-point being deter-

mined after the third crystallization onwards. Cholesterol acetate melts at 114.3° to 114.8° , whilst phytosteryl acetate melts at 125.6° to 137° according to the source from which it is obtained. If the crystals melt at 116° , vegetable oil is probably present; if at 117° or over, the presence of a vegetable oil is certain.

The Reichert Value.—The Reichert (or Reichert-Meissl) value indicates the number of c.c. of decinormal potash solution requisite for the neutralization of that portion of the soluble volatile fatty acids obtained from 2.5 grms. (or 5 grms.) of the fat when saponified and distilled by Reichert's method.

The Reichert value refers to 2.5 grms. of the fat, whereas the Reichert-Meissl or Reichert-Wollny value refers to 5 grms. of the fat.

Five grms. of the fat are accurately weighed into a flask of about 200

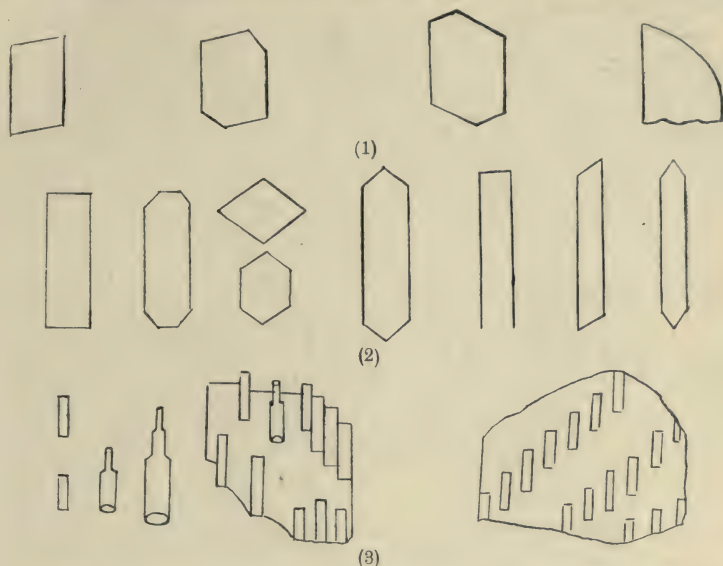


FIG. 57.—(1) Cholesterol; (2) Phytosterol; (3) Mixture of both.

c.c. capacity. About 2 grms. of stick potash and 50 c.c. of 70 per cent alcohol are then added, and the oil is saponified on a water bath, and the alcohol driven off completely. The soap is dissolved in 100 c.c. of water, and 40 c.c. of 10 per cent sulphuric acid added to the liquid (cold). A few small pieces of pumice stone are added to prevent "bumping".

Distil from the flask through a Liebig condenser, placing a safety bulb between the flask and condenser so as to avoid spurting, and collect 110 c.c., which should take about 1 hour to come over. One hundred c.c. of this are filtered and titrated with decinormal potash using phenolphthalein as indicator. The value so obtained is multiplied by 1.1 and this, in c.c. of potash solution gives the Reichert-Meissl value. This is not exactly double the Reichert value, but is usually about equal to the

Reichert value multiplied by 2.2. Filtration of the distillate is necessary, since a certain amount of volatile insoluble fatty acids are distilled over.

This process being an empirical one, requires careful attention to the exact details which should not be allowed to vary at all. (For further details see under butter.)

Refractive Values.—The determination of the refractive index is often a matter of importance with fats and oils, but it is usual to use an instrument which is not graduated in absolute indices, but in arbitrary degrees, when the value is returned as the "refractometer number". This is determined on the Zeiss-Abbé instrument, which is that found in most laboratories, and is known as the "butyro-refractometer". In quoting figures, the instrument should be quoted, as the values for a Zeiss-Abbé instrument differ materially from those of the Jean-Amagat refractometer.

For further details see under butter (p. 96).

The following table gives the limits of the usual values determined analytically for the commoner adulterants of some of the fatty oils. It is to be remembered that many of these oils are themselves edible, and there is no reason that they should not be sold, so long as they are properly labelled or described:—

	Specific Gravity.	Refractive Index.	Sap. Value.	Iodine Value.	Refract. No.
Poppyseed	0.923 to 0.927	1.458 at 60°	190 to 197	130 to 145	63 to 64 at 40°
Sunflower	0.927 „ 0.926	1.461 „ 60°	193 „ 196	120 „ 132	72 „ 73 „ 25°
Maize	0.921 „ 0.926	1.4762 „ 20°	188 „ 193	110 „ 130	69 „ 20°
Cottonseed	0.922 „ 0.926	1.4740 „ 20°	193 „ 196	108 „ 112	67 „ 69 „ 20°
Sesame	0.923 „ 0.925	1.475 to 1.477 at 15°	189 „ 194	103 „ 114	68 „ 25°
Arachis	0.917 „ 0.921	1.4550 at 60°	190 „ 196	84 „ 102	66 „ 68 „ 25°
Mustard	0.915 „ 0.920	1.4750 „ 15°	170 „ 174	95 „ 110	58 „ 60 „ 40°
Rapeseed	0.915 „ 0.917	1.4725 to 1.4758 at 15°	170 „ 180	95 „ 105	68 „ 25°
Cocoanut	0.911 „ 0.913	1.4400 „ 1.4420 „ 60°	245 „ 268	8 „ 10	34 „ 40°

THE FIXED OILS, FATS, AND WAXES OF THE BRITISH PHARMACOPŒIA.

ADEPS LANÆ.

Adeps Lanæ, or wool "fat" is described in the Pharmacopœia as the purified cholesterin fat of sheep's wool. It is stated to be a yellowish, tenacious, unctuous substance, almost inodorous; melting at 40° to 44.4° C., readily soluble in ether or chloroform, sparingly so in alcohol. One grm. should dissolve almost completely in 75 c.c. of boiling 90 per cent alcohol, the greater part separating in flocks on cooling; it should not yield more than 0.3 per cent of ash, and this should not be alkaline. It should not contain more than 0.28 per cent of free acids calculated as oleic acid. A solution in chloroform poured carefully into sulphuric acid acquires a purple-red colour. Heated with caustic soda solution no odour of ammonia should be evolved.

Anhydrous wool fat—or wool wax as it is more properly termed—is the natural grease extracted from the sheep's wool, purified and freed from fatty acids. Its extensive use depends on the fact that it forms an emulsion with 75 per cent of its weight of water, which is readily absorbed by the human skin, so that it forms a useful vehicle for certain forms of medication.

Wool wax consists of a complex mixture of esters and free alcohols. Amongst the alcohols, cholesterol and isocholesterol are the principal. Pure wool wax, freed from free fatty acids, should have the following characters:—

Specific gravity at 15°	0.940 to	0.950
" " " 10°	0.899 "	0.908
Melting-point	35°	45°
Saponification value	98	105°
Iodine value	20	30
Fatty acids	50	60 per cent
Alcohols (determined as unsaponifiable matter)	40	50 "

ALMOND OIL.

The oil expressed from the bitter or sweet almond is described officially as a pale yellow nearly inodorous oil with a nutty taste. Its specific gravity is given as 0.915 to 0.920, and it should not congeal until cooled to nearly -4° F. If 2 c.c. of the oil be shaken with 1 c.c. of fuming nitric acid and 1 c.c. of water, a whitish, not brownish-red, mixture should be formed, which after standing for six hours at 50° F. should separate into a solid white mass and a nearly colourless liquid. (Absence of peach kernel and other fixed oils.)

Almonds yield from 35 to 45 per cent of fixed oil, which consists essentially of glycerides of oleic and other liquid unsaturated fatty acids. No solid fatty acids—or not more than traces—are present in the oil. The usual adulterants of this oil are the fixed oils of the apricot and peach kernel, and of recent years a good deal of hazel nut oil has been used for the purpose of sophistication. From time to time other oils such as arachis, sesame, and olive oils have been used but these are not commonly met with.

Pure almond oil should have the following characters:—

Specific gravity at 15°	0.914 to	0.920
Saponification value	189	196
Iodine value	96	104
Refractive index at 15°	1.4710	1.4728
Butyro-refractometer No. at 15°	70	71
Melting-point of fatty acids	13°	14°
Solidifying point	" "	9.5	11.5°
Neutralization value of " (per cent KOH)	20.4	

Hazel nut oil may be readily recognized by the characteristic taste of the hazel nut. The iodine value of this oil is about 84 to 88 so that any considerable quantity will be indicated by a reduced iodine number. The fatty acids of hazel nut oil melt at from 19° to 25°, so that this figure will be raised if much of this oil is present.

Poppy seed oil has been found in a number of samples by the author during the past few years. This will be indicated by a higher

specific gravity, a higher refractive index, and a higher iodine value, that of poppy seed oil being from 135 to 140.

Practically no tests exist which will definitely prove the presence of apricot or peach kernel oils, except, to some extent, colour reactions. The official nitric acid test (see above) is a useful one, as both these adulterants give a yellow to red-brown fatty mass, when shaken with the acid.

Bieber's test is also fairly reliable. It consists in shaking 5 volumes of oil with one volume of a mixture of equal parts by weight of concentrated sulphuric acid, fuming nitric acid and water. Pure almond oil does not change colour, while apricot kernel oil gives a pink colour, and peach kernel oil a faint pink colour after standing for some time.

Figures have been from time to time published to show that the usual quantitative determinations may be of assistance in discriminating between pure almond oil and mixtures with peach or apricot kernel oil, but from the experience of a very large number of samples, the author has no hesitation in saying that these figures overlap so much that they are perfectly useless for the purpose of detecting these two oils.

Lewkowitsch ("Analyst," xxix, 105) gives the figures on opposite page, for almond, peach, and apricot kernel oils:—

CROTON OIL.

This oil is expressed from the seeds of *Croton tiglium*. The specific gravity is officially given as 0.940 to 0.960. The oil should be soluble in absolute alcohol, ether, and chloroform. An alcoholic solution should not redden litmus. If 2 c.c. be shaken with 1 c.c. of fuming nitric acid, and 1 c.c. of water, the mixture should not solidify, but only thicken slightly after standing for two days (absence of other non-drying oils).

Croton seeds contain about 55 per cent of fixed oil.

The oil contains glycerides of various fatty acids, amongst which are the lower fatty acids, such as formic, acetic, butyric, etc., so that a high Reichert value is always found. A small quantity of a resinous matter, probably of a lactone nature, exists, and is probably the purgative principle of the oil.

The statement which is contained in the Pharmacopœia that croton oil is soluble in absolute alcohol requires some qualification. According to Lewkowitsch, this is only true where the oil has been extracted from the seeds by alcohol. It is only true for expressed oils if less than an equal volume of alcohol be used: more alcohol at once causes turbidity.

Pure croton oil has the following characters:—

Specific gravity at 15°	0.937 to 0.943
Refractive index at 15°	About 1.4770
Saponification value	197 to 215
Iodine value	101 „ 112
Butyro-refractometer No. at 40°	67 „ 69
Melting-point of fatty acids	About 22°
Solidifying point of fatty acids	18° „ 19°
Neutralization value of fatty acids	20 „ 20.5 (per cent KOH)
Reichert-Meißl value	12 „ 14
Acetyl value	25 „ 36

Description of Oil.	Specific Gravity at 60° F. (Water 60° F. = 1).	Saponification Value.	Iodine Value.	Butyro-Refractometer at 40° C.	Acid Value.	FATTY ACIDS.		Bieber's Test.
						Neutralization Value.	Saponification Value.	
Almond oils, expressed from :—								
1. Valencia sweets . . .	0.91995	207.6	99.4	57.5	5.16	207.8	207.6	Colourless.
2. Blanched Valencia sweets . . .	0.9182	191.7	103.6	57.5	2.9	196.4	201.7	Colourless.
3. Sicily sweets . . .	0.9178	183.3	100.3	57.0	0.79	198.8	202.2	Colourless.
4. Mazagan bitters . . .	0.9180	188.6	102.5	56.5	3.1	196.8	203.1	Colourless.
5. Small Indian almonds . . .	0.91907	189.2	96.65	57.0	2.9	195.8	200.7	Colourless.
6. Mogador bitters . . .	0.9183	194.98	104.2	57.0	1.3	197.1	203.2	Colourless.
7. Peach kernel oil . . .	0.9198	191.4	95.24	57.5	3.0	196.8	205.0	Colourless at first then pink.
8. Apricot kernel oil . . .	0.9200	192.4	107.4	58.0	2.3	198.0	202.0	Pink coloration.
9. Apricot kernel oil from Mogador kernels . . .	0.9172	198.2	107.9	57.0	2.8	194.0	200.7	Slightly pink.
10. Californian apricot kernel oil . . .	0.92026	190.3	108.7	58.0	1.2	197.8	202.8	Very slightly pink.

This oil (which is not a non-drying oil, as would be indicated by the wording of the Pharmacopœial monograph, but a semi-drying oil) is not often adulterated. Most other fixed oils are revealed by a lowered specific gravity, and a reduced Reichert value. Castor oil is the only adulterant met with by the author, and this would be detected by a high acetyl value, a lower Reichert value, and a higher specific gravity.

LINSEED OIL.

Linseed oil is expressed from the seeds of *Linum usitatissimum*, which yield about 35 per cent of the oil, the pressed cake retaining about 10 per cent. Linseed oil is one of the most typical of the drying oils, and is used for technical purposes to a very large extent, its use in medicine being very small. For technical purposes valuations of the oil are required which are not necessary when dealing with the oil from a pharmaceutical point of view.

The only standards given in the Pharmacopœia are that the specific gravity should be from 0.930 to 0.940: the oil should be soluble in 10 parts of 90 per cent alcohol, and in turpentine. It gradually thickens by exposure to the air. It does not congeal above -20°C .

Linseed oil is, even when generally accepted as pure, a mixture of the oil from linseed with that from a small quantity of hemp or rape seed. This is due not to deliberate adulteration, but to the fact that the seeds are more or less accidentally mixed, owing to the fact of the proximity of the plants, which grow together in certain districts.

This oil consists of glycerides of palmitic and myristic acid (10 per cent) but principally of the glycerides of liquid fatty acids of which the principal are linolic, linolenic and isolinolenic acid, all being highly unsaturated. Pure linseed oil should have the following characters:—

Specific gravity at 15°	0.930 to 0.941
Refractive index at 15°	1.4830 „ 1.4845
Saponification value	190 „ 196
Iodine value	172 „ 192
Butyro-refractometer No. at 20°	84 „ 85.5
Melting-point of fatty acids	19° „ 23°
Solidifying point of fatty acids	13° „ 16°
Neutralization value of fatty acids	19.5 „ 20 (per cent of KOH)
Unsaponifiable matter	Under 1 per cent

If a sample complies with the above figures it is practically certain to be pure. The iodine value is characteristic, and if this falls below 170 the oil should be condemned. The only adulterations met with to any extent are those with mineral and rosin oils, the latter especially. Mineral oils will be indicated by the low specific gravity, the low iodine value, and the high unsaponifiable matter. Rosin oil will raise the specific gravity, and lower the saponification value, at the same time raising the amount of unsaponifiable matter. It is also dextrorotatory, by which feature it can be detected if present in any quantity. The amount of unsaponifiable matter however is the best criterion of the presence of these adulterants. Rosin oil may also be

detected in the following manner: Warm 5 c.c. of the oil with 10 c.c. of 90 per cent alcohol. When cold separate the alcoholic liquid, and evaporate the alcohol. Dissolve the residue in a few c.c. of acetic anhydride, and carefully pour on to the surface of the liquid a few drops of cold 50 per cent sulphuric acid. A fine violet colour, which is transient, results if rosin oil be present.

Most other oils if present will be revealed by the failure of the oil to comply with the standards above given. But as linseed oil is a cheap oil, most other oils are precluded from use as adulterants.

COD LIVER OIL.

Cod liver oil is required by the Pharmacopœia to have been freed from solid fat by filtration at about -5°C . The oil should be obtained from the livers of the codfish only, and is required to have the following characters:—

Specific gravity at 15° 0.920 to 0.930: no solid fat should separate by exposing the oil to a temperature of 0°C . for two hours: a drop of sulphuric acid added to a few drops of the oil on a porcelain slab develops a violet coloration: when nitric acid is carefully poured into some of the oil contained in a test tube, a precipitate of coagulated albumen should be formed at the surface of contact of the two liquids.

Cod liver oil, to be fit for medicinal purposes, should be prepared by steaming the livers within twenty-four hours after the fish are caught. The pale cod liver oil of pharmacy results by this treatment. When the fishing boats are unable to come ashore quickly, the fish are killed on board, and the livers stored. These may be brought to shore after several days or a week or more when they are often in a more or less decomposing condition. The oil obtained from these livers may be a pure cod liver oil but it is dark brown, and has an objectionable odour, and is only fit for veterinary purposes. At one time there was believed to be a great difference between the Norwegian cod liver oil, and the Newfoundland oil. This difference probably was due to the fact that other livers were formerly used in the preparation of the Newfoundland oil, and the livers were not pressed in a sufficiently fresh state. To-day, however, the difference is somewhat sentimental, as Newfoundland oil can be obtained of the highest grade, and perfectly pure. Oil of high grade is also made now on the East Coast of Scotland. The standards of the British Pharmacopœia are totally inadequate to discriminate between cod and many other liver oils. Indeed few fatty oils give so much difficulty to the analyst as this one, and in some cases it is impossible to decide whether an oil is pure or not.

The specific gravity given in the Pharmacopœia is common to a whole group of liver oils: the sulphuric acid colour reaction is equally common to a number of liver oils; and the nitric acid test for albumen is quite unreliable. New standards for this oil are therefore obviously required. A genuine cod liver oil should have the following characters:—

Specific gravity at 15°	0.920 to 0.930 (rarely up to 0.932).
Refractive index at 15°	1.4800 to 1.4825.
Saponification value	180 to 190 (rarely a little higher or lower).
Iodine value	158 to 168.
Butyro-refractometer No. at 15° .	81 to 86.
Neutralization value of fatty acids .	19.7 to 20.3.
Mean molecular weight of fatty acids	About 290.
Melting-point of fatty acids . . .	21.25.
Iodine value of fatty acids	165 to 172.

A properly-prepared cod liver oil will contain no glycerides of volatile fatty acids, and will never give a Reichert value of more than 1. Any higher value than this indicates the decomposition of the livers used in preparation of the oil.

The amount of unsaponifiable matter present in this oil is a very important determination. Genuine cod liver oil contains a little cholesterol, but in medicinal oils the total amount of unsaponifiable matter rarely exceeds 1.0 per cent, often being less than 0.5 per cent. Certainly any higher amount than 1.5 per cent should be condemned, as this is almost certainly due to the presence of other oils, of which the most usual is shark liver oil which usually contains as much as 5 to 8 per cent of unsaponifiable matter.

The amount of free fatty acids is important, and oils for medicinal use should not contain more than 0.6 per cent, or at most 0.8 per cent of free acids calculated as oleic acid. Higher values point to crude unrefined oils.

Apart from the adulteration with other fish oils which have very similar analytical values, cod liver oil is sometimes—although rarely—adulterated with vegetable oils. This is, as a rule, at once indicated by the lower iodine value. In cases of doubt the phytosteryl acetate test may be applied (see p. 630).

Numerous colour tests have been recommended for this oil. Most of these are absolutely useless, but the following are useful within certain limits :—

If one part of the oil be dissolved in five of carbon disulphide, and a few drops of concentrated sulphuric acid added, a fine blue colour will result, which is more purple if the oil is rancid. This colour test is a general one for liver oils, and not restricted to cod liver oil. It is not yielded by oils from other parts of the fish, such as blubber oil. The following is also a general test for liver oils :—

One gram of the oil is dissolved in 5 c.c. of chloroform in a test tube, and shaken with 2 c.c. of freshly prepared solution of phosphomolybdic acid. A blue ring is formed at the zone of contact of the liquids, in the presence of a liver oil.

Except in rare instances, where perhaps the oil has been exposed to the influence of air and light for some time, the following reaction is yielded by pure cod liver oil (but other oils may be in admixture with the cod liver oil without interfering with the reaction). If 10 drops of nitric acid be stirred for a minute with 5 c.c. of cod liver oil on a white tile, a pale rose colour results, which after standing becomes pale yellow. In the presence of some liver oils, the colour will be deep red, very soon changing to a dirty brown instead of a pale yellow. This reaction is only of value if a positive result is obtained.

The following values are those (Lewkowitsch) of some of the liver oils which are used for adulterating cod liver oil:—

Liver Oil from	Specific Gravity.	Saponification Value.	Iodine Value.	Acid Value.	Unsapon. Per cent.
Skate	0.9307 (at 15° C.)	185.4	157.3	—	0.97
Tunny	—	—	155.9	0.2 to 34	1.0 to 1.8
Haddock	0.9298 (at 15° C.)	188.8	154.2	—	1.1
Coal fish	0.925 (at 15° C.)	177 to 181	123 to 137	1.26 to 1.68	—
	0.9272 (at 15° C.)	186.1	137 to 162	7.2; 21.6	—
	0.9200 (at 15° C.)	184.1	139.1	2.8	6.52
Ling	—	—	132.6	10.9	2.23
Shark (Arctic)	0.9163 (at 15° C.)	161.0	114.6	—	10.2
" "	0.9105 to 0.9130	146.1 to 148.5	111.9 to 114.9	2.6 to 6.2	20.8 to 21.5
" (Japan)	0.9156, 0.9177	163.4, 163.5	128.3, 136	0.88, 1.5	14.4, 21.5
" "	0.9158	157.2	90	—	—
Ray	0.9280 (at 15.5° C.)	—	—	—	—
Hake	0.9270 (at 15.5° C.)	—	—	—	—

The interesting figures on page 640 are due to Barclay, and represent a number of samples of cod liver and other fish oils.

OLIVE OIL.

This oil has already been dealt with under foods (see page 111).

CASTOR OIL.

This oil is required by the British Pharmacopœia to have a specific gravity between 0.950 and 0.970: to be soluble in one volume of absolute alcohol, and in 5 volumes of 90 per cent alcohol. Equal volumes of castor oil and petroleum spirit are stated not to yield a clear mixture at 15.5°, but in the presence of other fixed oils the mixture is clear (as a matter of fact, the amount of other fixed oils present materially influences this test). Another official test is given which requires that 3 c.c. of the oil dissolved in 3 c.c. of carbon disulphide should not become brown when shaken with 1 c.c. of sulphuric acid. This test is quite incorrect, and the author has never met with a sample which literally answers it.

Castor oil consists principally of the glycerides of two or more unsaturated hydroxy acids, ricinoleic and isoricinoleic acids $C_{18}H_{34}O_3$.

The glycerides of stearic and dihydroxystearic acids are also present to a small extent, as well as traces of other glycerides.

There are numerous grades of castor oil, the quality employed in medicine being white or at most pale yellow in colour, and usually being a cold pressed oil.

SAMPLES OF CODLIVER OIL.

	Norwegian.				Newfoundland.				Origin Unknown.		
	A	B	C	D	E	F	G	H	J		
<i>Oil.</i>											
Specific gravity, 15.5° C.	0.928	0.928	0.928	0.928	0.926	0.927	0.928	0.927	0.928		
Zeiss butyro-refractometer at 25° C. . .	80.0	79.7	79.7	79.0	76.3	79.0	80.0	77.3	80.0		
Zeiss butyro-refractometer at 40° C. . .	71.0	70.7	70.3	70.3	67.7	70.0	71.0	68.7	71.0		
Rotation (200-millimetre tube)	-0.5°	-0.5°	-0.5°	-0.5°	-0.5°	-0.6°	-0.7°	-0.4°	-0.6°		
Iodine value	168	162	167	164	154	160	165	154	164		
KHO required per cent for free acid . .	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.1		
KHO total per cent	18.7	18.5	18.7	18.7	18.8	18.7	18.6	18.9	18.6		
Valenta test (°C.)	95	95	96	95	94	95	96	93	94		
Unsatifiable matter per cent	—	—	1.2	—	1.1	—	—	—	—		
<i>Fatty Acids.</i>											
Zeiss butyro-refractometer at 40° C. . .	57.0	56.7	56.7	55.7	53.7	56.3	57.0	54.7	56.7		

SAMPLES OF FISH AND OTHER OILS.

	Seal.	Shark.	Du-gong.	Had-dock.	Men-haden.	Whale.	Brus-mer.	Hoi.	Ling	Arachis.	Sesame.	Cotton-seed.
<i>Oil.</i>												
Specific gravity, 15.5° C.	0.925	0.962	0.919	0.934	0.931	0.917	0.923	0.919	0.923	0.916	0.922	0.923
Zeiss butyro-refractometer at 25° C. . .	72.7	87.3	60.3	84.0	80.7	65.0	75.0	73.7	74.0	63.7	68.0	68.7
Zeiss butyro-refractometer at 40° C. . .	64.0	77.7	52.0	74.3	71.3	56.0	66.3	64.7	65.0	55.3	59.7	60.0
Rotation (200-millimetre tube)	0°	-0.5°	-0.1°	-0.5°	-0.4°	-1.0°	-0.5°	-4.0°	-0.6°	0°	+0.9°	0°
Iodine value	132	142	69	179	174	94	138	124	133	84	106	112
KHO required per cent for free acid . .	0.5	2.2	0.5	0.6	0.5	0.4	0.1	0.1	0.1	0.3	0.2	0.1
KHO total per cent	19.4	6.0	20.2	19.3	19.3	18.8	18.3	16.9	18.8	19.1	19.2	19.6
Valenta test (°C.)	88	35	86	73	78	100	108	113	105	102	85	90
Unsatifiable matter per cent	1.0	84.0	0.9	1.0	0.6	1.0	—	—	—	—	—	—
<i>Fatty Acids.</i>												
Zeiss butyro-refractometer at 40° C. . .	49.7	—	37.7	60.7	57.3	43.3	53.3	52.7	52.3	42	46	46

A medicinal castor oil should not contain more than from 1 per cent to 2 per cent of free fatty acids calculated as oleic acid. It should have the following characters:—

Specific gravity at 15°	0.955 to 0.968
(the official limits are too wide)	
Saponification value	176 „ 186
Iodine value	82 „ 88
Acetyl value	145 „ 150
Refractive index at 15°	1.4790 „ 1.4810
Butyro-refractometer No. at 25°	77 „ 79
Optical rotation (100 mm.)	3° „ 5°
Solidifying point of fatty acids	3°
Mean molecular weight of fatty acids	290 to 303
Refractive index of fatty acids at 15°	1.4540 to 1.4558

Castor oil is distinguished from other fixed oils by its very high acetyl value, its optical activity, and its solubility in alcohol and petroleum spirit. Nearly every genuine sample will dissolve in 3 to 3.5 volumes of 90 per cent alcohol. The official test for this oil in reference to its solubility in petroleum spirit is misleading. It is true that 1 per cent of castor oil is not soluble in petroleum spirit, but castor oil dissolves its own volume of the spirit, so that mixtures in equal parts give a clear solution, but if more than 1 volume of the spirit be employed with 1 volume of the oil a turbid mixture results. Most samples also give a clear solution with one and a half times their volume of kerosene, but not with larger quantities. Small quantities of other fatty oils, the amount depending on the nature of the oil, cause the oil to lose the characteristic insolubility, and such adulterated oils are soluble in all proportions in petroleum spirit.

An acetyl value below 140 is strong evidence of adulteration, as no oil, except perhaps grape seed oil (which is not used as an adulterant) has an acetyl value anywhere near that of castor oil.

The sulphuric acid test of the Pharmacopœia must be ignored as it is quite incorrect.

OIL OF THEOBROMA.

This has been dealt with under cocoa (see page 26).

BEESWAX.

Beeswax is official both as white and yellow wax (*Cera alba* and *Cera flava*). The official tests for both varieties are identical, and are as follows:—

The wax should have a specific gravity at 15° of 0.960 to 0.970; should melt at 62.5° to 63.9°: it should not yield more than 3 per cent to cold 90 per cent alcohol: it should not yield anything to water or boiling solution of caustic soda, the liquids filtered after such treatment not giving any precipitate when acidified by HCl (absence of fatty acid, Japan wax and resin): 5 grms. should require not less than 1.6 c.c. of normal alkali to neutralize the free fatty acids present, and between 6.2 and 6.8 c.c. of normal alkali to saponify the esters present. After heating 5 grms. with 25 grms. of sulphuric acid to 160° C. for fifteen minutes and diluting the mixture with water no solid wax

should separate (absence of paraffin wax). It should yield no reaction for starch.

Beeswax consists essentially of a mixture of free cerotic acid $C_{26}H_{52}O_2$ and myricin (myricyl palmitate) $C_{30}H_{61}O \cdot CO \cdot C_{15}H_{31}$.

It also contains small quantities of other free acids, traces of free myricyl and ceryl alcohols, and other bodies which are not well understood. A small amount of hydrocarbons also exists in beeswax.

Beeswax is very often adulterated, the common adulterants being paraffin or ceresin wax, Japan wax, tallow, stearic acid, resin, and insect wax.

Carnauba wax used to be a common adulterant, but its price is now too high for it to be so employed.

The usual adulterants to-day, are mixtures of some of the above, so blended as to give analytical results very similar to those of pure beeswax. Pure beeswax should have the following characters:—

Specific gravity at 15°	0.962 to 0.970
Specific gravity „ 100°	0.818 „ 0.824 (water 15° = 1)
Melting-point	60.5° „ 64°
Acid value	18 „ 21.2 (rarely as low as 16.8)
Ester value	72 „ 81
Saponification value (total)	90 „ 99 (rarely 88)
Iodine value	8 „ 12

In judging the above figures it must be remembered that a large number of samples of Indian beeswax give figures which are far outside the above limits. Whether these samples are due to abnormal conditions or whether they are regularly adulterated with some unknown adulterant is not yet definitely known. At all events such beeswax cannot safely be employed in medicine. The specific gravity at 15° is best taken by carefully melting the sample and cutting small portions with a sharp cork borer, and mixing methylated spirit and water of various strengths, so that in one mixture (at 15°) the fragments just float, whilst in another containing a trace more of the alcohol they just sink: the specific gravities of the liquids are taken, and the mean of the two is taken as that of the wax. Care must be taken that no air bubbles are adherent to the wax.

The ratio of the ester value to the acid value, the “ratio no.” as it is called, is fairly constant, and will be found to vary between 3.5 and 4.1 usually about 3.7. Most adulterants will upset this ratio.

The following figures are those of some of the commoner adulterants of beeswax:—

	Acid Value.	Ester Value.	Ratio Number.
Japan wax	19 to 22	200 to 210	About 11
Chinese wax	Traces	78 „ 82	Very high
Spermaceti	„	130 „ 135	„
Myrtle wax	2 to 5	205 „ 210	„
Tallow	3 „ 5	190 „ 198	„
Stearic acid	190 „ 200	None	Very infinitesimal.
Resin	140 „ 165	20 to 30	$\frac{1}{8}$ to $\frac{1}{6}$
Paraffin and ceresin	—	—	—

When mixtures for adulteration have been prepared so as to give correct acid ester and ratio numbers, hydrocarbon wax and stearic acid are usually present together with a wax, such as Japan wax, with a high ester value.

These bodies must therefore be searched for.

Japan wax will be indicated by the presence of glycerin. If this has to be determined the following process is the best. Twenty grms. are saponified in the usual way, and the alcohol evaporated, the resulting mass boiled with water, and excess of sulphuric acid added. The separated waxy matter is filtered off, and washed with boiling water, and the glycerin in the filtrate determined by Lewkowitsch's process, which is as follows:—

The filtrate is neutralized with an excess of barium carbonate and boiled down on the water bath until most of the water is driven off. The residue is exhausted with a mixture of ether and alcohol, and the ether-alcohol driven off for the most part by gently heating on the water bath, and the residue dried in a desiccator and weighed. It is not necessary to dry until constant weight is obtained, since the glycerol is determined in the crude product by the acetin method.

This process is based on the conversion of glycerol into triacetin when concentrated glycerol is heated with acetic anhydride. If the product of this reaction is then dissolved in water, and the free acetic acid has been carefully neutralized with alkali, the dissolved triacetin can be easily estimated by saponifying with a known volume of standard alkali and titrating back the excess. The solutions required are:—

1. Half normal or normal hydrochloric acid.
2. Dilute caustic soda, containing about 20 grms. of NaOH in 1000 c.c.
3. Its strength need not be known accurately.

3. A 10 per cent solution of caustic soda.

The estimation of the glycerol is carried out as follows:—

About 1.5 grms. of the crude glycerin weighed accurately are heated with 7 c.c. to 8 c.c. of acetic anhydride and 3 grms. of anhydrous sodium acetate for one and a half hours in a flask, of about 100 c.c. capacity, connected with an inverted condenser. The mixture is then allowed to cool a little, 50 c.c. of warm water are poured down through the tube of the condenser, and the acetin made to dissolve by shaking the flask; if necessary, the contents of the flask may be slightly warmed, but must not be boiled. As triacetin is volatile with water vapours, these operations must be carried out whilst the flask is still connected with the condenser. The solution is next filtered from a flocculent precipitate, containing most of the impurities of the crude glycerin, into a wide-mouthed flask of about 500 c.c. to 600 c.c. capacity, and the filtrate allowed to cool to the ordinary temperature. Phenolphthalein is then added, and the free acetic acid neutralized with the dilute caustic soda solution. Whilst running in the soda the solution must be agitated continually, so that the alkali may not be in excess locally longer than is unavoidable. The point of neutrality is reached when the slightly yellowish colour of the solution just changes into

reddish-yellow. If the solution is allowed to become pink, the point of neutrality has been exceeded, and a fresh test must be made; the excess of soda cannot be titrated back, as partial saponification of the acetin takes place in presence of the slightest excess of alkali. The change of colour is very characteristic, and is easily noticed after some little practice.

Twenty-five c.c. of the strong soda solution are now run in and the solution boiled for a quarter of an hour; the excess of soda is then titrated back with the standard acid. Side by side, operating in the same manner, 25 c.c. of the strong caustic soda are boiled and titrated with acid. The difference between the two titrations corresponds to the amount of alkali required for the saponification of the triacetin. From this the quantity of glycerol in the sample can be calculated, as shown in the following example: Suppose 1.324 grms. of the sample have been treated as described above. Let 25 c.c. of the strong alkali require 60.5 c.c. of normal hydrochloric acid, and let the number of c.c. required for titrating back the excess of soda in the sample be 21.5 c.c., then $60.5 - 21.5 = 39.0$ c.c. have been used. One c.c. of normal acid corresponds to $\frac{9.92}{39} = 0.03067$ gm. of glycerol. Hence the sample contained $0.03067 \times 39 = 1.1960$ grms. or 90.3 per cent of glycerol.

The percentage found is calculated to the crude glycerin obtained on saponifying the original quantity of 20 grms.

Added stearic acid or resin are indicated by a high acid value, and resin by a high iodine value. But if the acid value is adjusted by the presence of hydrocarbon wax, stearic acid may be detected by the following method: 1 gm. is boiled with 10 c.c. of 80 per cent alcohol. On cooling the filtered alcohol is poured into water. In the case of pure beeswax the liquid will remain clear or at most slightly opalescent. If stearic acid be present flocks of the acid will be precipitated, and rise to the surface.

An approximate determination of the stearic acid present may be made by boiling the wax with 90 per cent alcohol and titrating the filtered liquid with semi-normal alkali. This will include the free acids of resin if present. Paraffin and ceresin wax, if present in large quantity, will be revealed by the low acid and ester values, unless these have been adjusted by other adulterants. If so, the charring by sulphuric acid may be resorted to, or the actual amount of unsaponifiable matter determined. By the usual process the alcohols of beeswax will be returned as unsaponifiable matter. This will vary for pure waxes between 48 and 54 per cent, so that any considerable amount of hydrocarbons present will be detected. But the most accurate process is that of Buisine. About 5 to 10 grms. of the wax are heated with potash-lime to 250° C., and the mass powdered and extracted in a Soxhlet tube with petroleum ether. The extract is filtered if necessary, the solvent evaporated and the residue dried and weighed. Genuine beeswax yields from 12.5 to 16.5 per cent of hydrocarbons under these circumstances, so that the presence of more than 5 per cent of paraffin wax will be indicated.

SPERMACETI.

Purified spermaceti is described in the Pharmacopœia as a concrete fatty substance obtained mixed with oil from the head of the sperm whale, *Physeter macrocephalus*. As a matter of fact it is obtained both from the head and the blubber of the sperm whale, and also from the bottle-nose whale, *Hyperoödon rostratus*, and possibly from other allied species.

The official requirements for this substance are that it should melt at 46° to 50° C., that it should be reducible to powder by the aid of a little alcohol, and that it should be insoluble in water, nearly insoluble in cold alcohol but soluble in ether, chloroform, boiling alcohol and in fixed and volatile oils. The absence of stearic acid is provided for by the following test. When boiled with 90 per cent alcohol, and the liquid cooled and filtered, the filtrate should not give a flocculent precipitate when added to water. The following test is given to limit the free acidity: 0.2 gm. is dissolved in 20 c.c. of hot alcohol (90 per cent), and 2 drops of phenol-phthalein solution added. One drop of decinormal soda solution should produce a permanent red colour.

Spermaceti consists chiefly of acetyl palmitate (acetin) $C_{16}H_{33}O$. $CO \cdot C_{15}H_{31}$.

A small quantity of other esters is present, and also a small amount of free acetyl alcohol.

Spermaceti is rarely adulterated, as its characteristic crystalline appearance is destroyed by nearly every possible adulterant. Pure spermaceti should have the following characters:—

Specific gravity at 15°	. . .	0.950 to 0.960
Specific gravity at 100°	. . .	0.808 „ 0.816 (water at $15^{\circ} = 1$)
Melting-point	. . .	44 to 48°
Iodine value	. . .	3 „ 4.5
Saponification value	. . .	125 „ 135
Fatty acids	. . .	51 „ 54 per cent
Alcohols	. . .	49 „ 52 „

The free acids and the alcohols are determined on the portion of the sample used to determine the saponification value. The alcohols are extracted from the saponification liquor, after driving off the alcohol by extraction with ether, as in the determination of unsaponifiable matter. From the aqueous liquid the fatty acids are precipitated by hydrochloric acid, collected, washed, dried, and weighed. The sum of the acids and alcohols will be more than 100 per cent as in the decomposition water is taken up.

The slightly variable figures for the iodine value and the melting-point of a spermaceti are due to a varying amount of sperm oil which is left in the purified spermaceti. Pure spermaceti is practically neutral, and any excess of free fatty acids over 0.5 per cent will be due to careless preparation, or, more probably, to free stearic acid, which may be detected by the official test mentioned above. A small amount of sperm oil will raise the iodine value considerably, as the iodine value for the oil is over 80.

PETROLEUMS.

Three varieties of petroleum are official, the liquid, soft, and hard paraffins.

The official tests are sufficient to ensure their purity, but in case of any doubt, 5 grms. of the sample should be boiled with 20 c.c. of alcoholic potash (semi-normal) for half an hour. Not more than the slightest trace of alkali should be used in the process, otherwise fatty substances are present.

The following are the official tests:—

Liquid Paraffin.—A colourless, odourless, and tasteless hydrocarbon liquid, free from fluorescence. It should not boil below 360° C. Specific gravity 0.885 to 0.890.

Three c.c. heated with an equal volume of sulphuric acid to 100° C., for ten minutes with frequent agitation should not colour the acid more than a pale brown (many samples do not pass this somewhat too stringent test).

Alcohol when boiled with the sample should not colour blue litmus paper red. A mixture of 4 c.c. of the sample and 2 c.c. of absolute alcohol, and 2 drops of a saturated solution of lead oxide in 20 per cent solution of caustic soda, should remain colourless when kept at 70° C. for ten minutes (absence of sulphur compounds).

Soft Paraffin.—This may be either white or yellow. It should be free from acidity and alkalinity, and free from unpleasant odour and taste when warmed to 120° F. Its specific gravity at its melting-point should be 0.840 to 0.870 (water at 15.5° presumably being 1). It melts at 35.5° to 38.9° C., or even somewhat higher, and gives no acrid vapour when volatilized, and leaves no ash. It is insoluble in water, slightly soluble in absolute alcohol, and freely soluble in ether, chloroform, and benzol. On treatment with boiling 20 per cent caustic soda solution (aqueous) the separated aqueous liquid should yield no precipitate on the addition of excess of acid (absence of fixed oils, fats and resin).

Hard Paraffin.—This should be colourless, inodorous, and tasteless. Its specific gravity is 0.820 to 0.940. It is insoluble in water, slightly soluble in absolute alcohol, and almost entirely soluble in ether. An alcoholic solution should not redden litmus. It melts at 54.5° to 57.2°, and leaves no ash when burned.

LARD.

This fat is official in the Pharmacopœia, and has been dealt with under Foods (see page 106).

SUET.

This fat is official in the Pharmacopœia, and has been dealt with under Foods (see page 111).

SOAPS.

Three varieties of soap are official, curd or animal soap; hard (olive oil) soap; and soft (olive oil) soap.

Curd Soap or *Sapo Animalis*, as it is officially termed, is directed to be a soda soap made with purified animal fat consisting principally

of stearin. It is to contain about 30 per cent of water. The official standards for this soap are as follow:—

White or pale grey in colour; becomes horny and pulverizable when kept in warm dry air. It is soluble in 90 per cent alcohol, sparingly so in cold, but easily in hot, water. If 5 grms. of the dried and powdered soap be digested with boiling 90 per cent alcohol, and filtered while hot, and the filter washed with a little more alcohol, the filtrate should not give a red or pink colour with phenol-phthalein. And if the filter be then washed with hot water the washings shall not require more than 3 c.c. of decinormal sulphuric acid to discharge the red colour imparted to phenol-phthalein. It should not impart a greasy stain to unglazed paper. The ash yielded on incineration does not deliquesce. It should contain about 30 per cent of moisture.

A well-made animal soap should contain about 60 to 62 per cent of fatty anhydrides and 7.2 to 7.5 per cent of alkali calculated as Na_2O .

The fatty anhydrides may be determined by dissolving 5 grms. of soap in hot water, decomposing with HCl , and adding 3 grms. or thereabouts of paraffin wax accurately weighed, to the hot liquid. The fatty acids and wax solidify and can be removed in a cake from the liquid. They are melted with distilled water, well stirred in order to wash them, and again separated. This cake is removed, adherent moisture removed by filter paper, and the cake then dried at 105° . The weight less the weight of wax added gives the fatty acids. From this an average of 7 per cent of the weight must be deducted to convert into fatty anhydrides, two molecules of the acids losing one of H_2O in becoming anhydrides.

Sapo durus is to be made from olive oil. The tests for free alkalis, mineral matter, and moisture are identical with those for *Sapo animalis*.

No official method of deciding whether the oil used for its manufacture is olive oil or not is given. The soap (about 20 grms.) should be dissolved in hot water and the fatty acids liberated by the addition of hydrochloric acid, the free fatty acids separated, washed twice to render them free from HCl , and dried. They should then have the characters given under olive oil (see page 111) for the fatty acids of olive oil. The tests there described for arachis, sesame and cotton oil may be applied to the free fatty acids. A properly made hard soap should contain about 30 per cent of water, 60 to 62 per cent of fatty anhydrides, and 7.2 to 7.5 per cent of alkali calculated as Na_2O .

Sapo mollis is the potash soap made with olive oil. It is described as containing not more than 3 per cent of matter insoluble in warm 90 per cent alcohol; it must not contain more free alkali than that allowed by the tests given under *Sapo animalis* (see above). It yields a deliquescent ash, which should not afford any reaction for copper.

No limit for water is given, but a pure soft olive oil soap will usually contain about 48 per cent to 50 per cent of water, and 38 per cent to 40 per cent of fatty anhydrides. The ash should consist almost entirely of potassium carbonate, and should on titration with standard acid, yield results equivalent to from 6.5 per cent to 7 per cent of alkali calculated as K_2O . The fatty acids should be examined in the same manner as those of *Sapo durus*.

CHAPTER XII.

THE CHEMICALS OF THE PHARMACOPŒIA.

IN the present chapter, a number of the purely chemical substances included in the Pharmacopœia are dealt with merely in tabular form, the figures giving certain well-marked characters, and indications of probable impurities, etc. Others, especially when their examination involves something more than simple inorganic testing, are dealt with at greater length as their importance appears to justify.

The presence of small quantities of lead or arsenic in chemicals has of late years attracted considerable attention, and it is probable that in the next edition of the Pharmacopœia, limits of such impurities will be fixed. The necessity of such limits has become obvious when it is remembered that the harmlessness of given quantities is often a matter of conflicting evidence in the police courts, and that convictions have taken place when, for example, cream of tartar has been contaminated with $\frac{1}{3}$ rd of a grain of lead per lb., whilst in another court, acquittal followed when there was over 1 grain per lb. The attention which has recently been paid to this matter justifies its full treatment in this chapter. At the same time attention may be called to the fact that, although no quantitative standards exist officially, in March, 1907, Dr. MacFadden reported to the Local Government Board (Reports of Inspector of Foods, No. 2, 14 March, 1907) on the question of lead and arsenic in citric and tartaric acids, and cream of tartar. The limits set out in that report, although not legal "standards" have sufficient weight to largely influence magisterial decisions in the case of either these or similar chemicals, and are therefore of much importance, at all events pending the issue of a new edition of the Pharmacopœia. The conclusion arrived at by the reporter was that less than 0.002 per cent of lead and 0.00014 per cent of arsenic (Ar_2O_3) would not be sufficient to justify the condemnation of such substances.

The Present Official Tests for Lead and Arsenic.—The tests for arsenic are not described in the monographs of the Pharmacopœia, but are grouped in Appendix III, pp. 418-9. They are as follows:—

ARSENICUM.

Hydrogen sulphide affords in solutions containing hydrochloric acid a yellow precipitate, soluble in solution of potassium hydroxide, potassium carbonate, ammonium hydrosulphide, and potassium hydrogen sulphite, and in solution of the official ammonium carbonate, but

re-precipitated on addition of hydrochloric acid. The precipitate is insoluble in the strongest hydrochloric acid.

Nascent hydrogen, generated by the interaction of zinc and diluted sulphuric acid, converts arsenium compounds into hydrogen arsenide. A cold porcelain tile held in the flame of this gas acquires a dark metallic deposit, which is readily dissolved by solution of chlorinated soda. The gas, when passed into excess of solution of silver nitrate, causes a black precipitate of silver, and the cautious addition of solution of ammonia to the supernatant liquid causes a yellow precipitate.

Hydrogen, generated by the interaction of zinc and solution of potassium hydroxide or sodium hydroxide, converts arsenium compounds into hydrogen arsenide. This gas gives a black stain to filtering paper soaked with solution of silver nitrate and placed as a cap over the tube in which the test is being performed. Hydrogen antimonide is not evolved from antimony compounds under similar circumstances. The operation should be performed in an atmosphere which is free from hydrogen sulphide.

Stannous chloride dissolved in a large excess of hydrochloric acid gives on boiling with a solution containing arsenium a brownish-black precipitate.

Bright copper foil precipitates arsenium from solutions acidulated by hydrochloric acid, and the arsenium may be volatilized by heat in an open tube, when it condenses, at some distance from the copper, as a white sublimate of characteristic octahedral crystals.

Arsenites.—Solutions of arsenites yield a yellow precipitate with solution of silver ammonio-nitrate.

Arsenates.—Solutions of arsenates yield a reddish chocolate precipitate with solution of silver ammonio-nitrate. Solution of magnesium ammonio-sulphate affords a white crystalline precipitate.

LEAD.

With lead some confusion exists. In the preface to the Pharmacopœia pp. xiii. to xiv. it is stated as follows: "The qualitative tests by which the basylous and acidulous radicals of ordinary salts are recognized, and by which common impurities are detected, instead of being many times repeated in the text, as in previous editions of the Pharmacopœia, are given once for all in an Appendix, the text simply stating the names of the radicals or other matters which should be present or absent respectively. Special tests or tests rarely employed, are still given in the text."

This raises a very important point in reference to many of the convictions obtained during the past few years for cream of tartar alleged to contain lead, so that it failed to correspond with the Pharmacopœial requirements. Under *acidum citricum*, a very delicate test for lead is specifically described in the monograph. It provides for an acid which shall not even darken when dissolved in ammonia and treated with H_2S . Under *acidum tartaricum*, the acid is directed to comply with the test for lead given under *acidum citricum*. But on referring to *potassii tartras acidus*, or purified cream of tartar, it

states that "It should yield no characteristic reaction with the tests for lead". These tests are, of course, those on pages 424 to 425 of the third appendix, and are as follows:—

"Hydrochloric acid affords, except in very weak solutions, a white precipitate, soluble in boiling water. The aqueous solution as it cools deposits the lead chloride in the crystalline form.

"Hydrogen sulphide, in not very strongly acid solutions, yields a black precipitate insoluble in dilute hydrochloric acid, solution of potassium hydroxide, and solution of ammonium hydrosulphide. It is decomposed by boiling with diluted nitric acid, being partly converted into soluble lead nitrate and partly into white insoluble lead sulphate and sulphur. Dilute sulphuric acid causes a white precipitate almost insoluble in water, and still less soluble in dilute sulphuric acid and in alcohol, but soluble in solution of ammonium acetate.

"Solution of potassium chromate produces a yellow precipitate readily soluble in solution of potassium hydroxide, in strong hot nitric acid, sparingly soluble in diluted nitric acid, insoluble in acetic acid.

"Solution of potassium hydroxide gives a white precipitate soluble in excess of the reagent but insoluble in solution of ammonia."

Not one of these tests is satisfactory for detecting small quantities of lead, except the hydrogen sulphide test, and this is vitiated in the case of cream of tartar, since it is directed to be applied in solution "not very strongly acid," and as cream of tartar is about 1 in 200, heavy traces of lead—certainly heavier than ought to be present—would fail to produce a black precipitate.

NON-OFFICIAL CONSIDERATIONS.

The report of Dunstan and Robinson to the Pharmacopœia committee of the General Medical Council, with possible modifications in cases where the requirements may be considered rather too stringent, will probably largely influence the question in the next edition of that work. The recommendations embodied in this report are as follows:—

TESTS FOR ARSENIUM.

The tests described on pp. 418 and 419 of the British Pharmacopœia, 1898, to be replaced by the following:—

Those drugs which are directed not to yield any characteristic reaction with the tests for arsenium should be proved to contain less than three parts of arsenium in one million parts of the drug (three parts of arsenium are equivalent to four parts of arsenious anhydride), except in the cases of *acidum citricum* and *acidum tartaricum*, which should be proved to contain less than one part of arsenium in one million parts of the drug; and in the cases of *acidum hydrochloricum*, *acidum nitricum*, and *acidum sulphuricum*, which should be proved to contain less than three-tenths of one part of arsenium in one million parts of the drug; and in the case of *liquor ammoniæ fortis*, which should be proved to contain less than one-tenth of one part of arsenium in one million parts of the drug.

The freedom of the drug from these quantities of arsenium is to be

proved by comparing the stain it yields when submitted to that one of the following tests suited to its nature with the stain yielded by liquor arsenici hydrochloricus suitably diluted and submitted to the same test.

Each reagent employed must contain less arsenium than the limit prescribed for it; allowance can be made, on the one hand, for an increase in the stain due to any minute quantities of arsenium (below these limits) contained in the reagents, and, on the other hand, for any diminution in the stain due to the process, by employing the same reagents in a similar manner when preparing the stain used as a standard for comparison.

The analyst should satisfy himself, especially in using the tests involving more than the simplest operations, that his method of procedure is capable of finding the arsenium, by first testing the drug with the addition of 3 c.c. of the diluted liquor arsenici hydrochloricus or of a solution of sodium arsenate of the same strength as regards arsenium, by the prescribed test. It will be found that in such cases the stain is not quite so deep as that obtained in the case of *water* and the easily soluble drugs.

Test A.

A solution of 4 grms. of the drug is to be prepared as described below, and it is to be diluted with *water* to a volume of 25 c.c. This solution is to be placed in a test-tube of about three-quarters of an inch (about 18 mm.) in diameter and 7 to 8 inches (18 to 20 cm.) in length. Fragments of granulated *zinc* are to be put into the test-tube until they reach to about two-thirds of the height of the liquid. Immediately after adding the *zinc* a small plug of *cotton-wool* is to be placed in the test-tube above the liquid, and then a plug of *plumbized cotton-wool*, so as to leave a short space between the two plugs, and a closely fitting cap formed of two *mercurialized test-papers* is to be fastened on; it must not be torn at all when fastened on the test-tube. The test is to be allowed to continue for two hours at least, and the test-paper cap is to be examined by daylight for a yellow stain. The test should be conducted in a place protected from strong light.

Ten c.c. of the liquor arsenici hydrochloricus are to be diluted to 75 c.c. (1 c.c. of the product contains 1 mg. of arsenium). Four c.c. of this solution are to be diluted, not more than a week or two before the test is made, to 1 litre (dilute solutions of arsenium have sometimes been found to give weaker reactions after keeping than when fresh). Each c.c. of this solution contains 4 one-thousandth parts of a mg. of arsenium, and is equivalent for purposes of comparison with 4 grms. of drug to 1 part per million, so that the yellow stain from 4 grms. of drug should be less than the yellow stain from 3 c.c. of this solution mixed with *water* and with 5 c.c. or other suitable quantity of *hydrochloric acid*, and diluted to 25 c.c. and tested in a similar manner and at the same time.

When the drug cannot be conveniently obtained dissolved in 25 c.c. of liquid, or when the liquid froths excessively, the experiment can be

conducted in a small flask, the stain being compared with a standard stain obtained from an equal volume of liquid in a similar flask having a mouth of the same diameter. The flask should be shaken occasionally to mix the liquid and prevent the heavy zinc-chloride solution from settling at the bottom.

The *mercurialized test-paper* cap is to be prepared by moistening two pieces of smooth white filter-paper placed together with a few drops of *test-solution of mercuric chloride* and drying them. Hydrogen arsenide produces a yellow stain on this test-paper, and thus shows the presence of arsenium in the drug. The stain may be examined the day after performing the test if exposure to light is avoided. Dampness of the paper diminishes the intensity of the stain produced. Light acts on the yellow stain, causing it to fade or turn grey; the action is only noticeable after a few days if the light is dull, but if the light is at all bright the action is rapid. A stain placed between glass plates and exposed to bright daylight fades considerably in an hour or two; without the glass plates it turns grey. The stain lasts longer in the dry air of a desiccator than in ordinary air. Access of ammonia must be avoided, as it turns the stain grey. Hydrogen antimonide produces an orange or grey stain; hydrogen phosphide and hydrogen sulphide also produce yellow stains, and any sulphur dioxide in the solution is changed into hydrogen sulphide by the action of the zinc.

The plug of *plumbized cotton-wool* is to be made of *cotton-wool* previously soaked in *solution of lead acetate* squeezed and dried. It is used in order to remove any traces of hydrogen sulphide, and the lower plug of *cotton-wool* is to prevent the spray from washing down the lead acetate into the liquid beneath. Sulphur compounds should be oxidized to sulphates when preparing the liquid for testing, and the *plumbized cotton-wool* plug should be relied on only to remove traces of hydrogen sulphide. A yellow stain due to sulphur, when cut out and treated with a few c.c. of *hydrochloric acid* disappears in less than ten minutes, and can thus be distinguished from a stain due to arsenium, which, when thus treated, changes to an orange colour and lasts for one or two hours.

As the rate of evolution of the hydrogen varies with different samples of zinc and with the temperature, the amount of *hydrochloric acid* used should be varied if necessary from 5 c.c. so that the effervescence may be brisk but not violent. If the effervescence is very soon over a further addition of *hydrochloric acid* can be made, to see if the stain becomes deepened by further evolution of hydrogen. By using large and long fragments of granulated *zinc* it can be made to extend high up in the tube without employing a great weight of it. Granulated zinc is liable to absorb sulphur compounds on its surface; it can be freed from these by washing with *hydrochloric acid* for a few seconds, and then with water, shortly before use.

In certain cases the oxidation of sulphur compounds in the solution to be tested can be effected by means of the treatment with bromine, and then with hydroxylamine hydrochloride, as described in Test B.

The presence of iron in the *zinc* or in the liquid must be avoided, as it diminishes the amount of hydrogen arsenide evolved, and nitrates

and other oxidizing agents must be absent, as they also diminish the intensity of the stain or prevent its formation.

The test can be simplified by omitting the use of the *plumbized cotton-wool* plug and other precautions against hydrogen sulphide, and inferring the absence of arsenium if no yellow stain is produced, and repeating the test with the proper precautions if a yellow stain is found.

Test B.

Four grms. of the drug are to be placed in a flask of about 60 c.c. capacity, together with 2 grms. of *potassium metasulphite* and 22 c.c. of a mixture of *hydrochloric acid* and *water*, in such proportions that after reacting there shall be hydrochloric acid solution approximately of the constant boiling strength—that is 20 parts of free hydrochloric acid to 80 parts of water. The 2 grms. of *potassium metasulphite*, together with 4.1 c.c. of *hydrochloric acid*, produce such acid; if the drug contains no water of crystallization, and yields no water or volatile acid or free organic acid by its reaction with hydrochloric acid, then 11 c.c. of *hydrochloric acid* and 7 c.c. of *water* will produce 18 c.c. of such acid, thus making 22 c.c. in all. If hydrochloric acid is decomposed and water or volatile acid or free organic acid produced, then more *hydrochloric acid* and less *water* must be used.

The flask is to be immediately attached to a condenser in the position suited for distilling, and having a receiver at the lower end. The internal diameter of the condenser-tube should not exceed 8 mm. The liquid is then to be heated gently for about one hour in order to reduce arsenic compounds to arsenious compounds; it is then to be distilled until about three-fourths of it have passed over. The distillate is to be partially neutralized with *strong solution of ammonia*, so as to leave about 4 c.c. or other suitable quantity of hydrochloric-acid solution of the constant-boiling strength unneutralized (1 c.c. of *strong solution of ammonia* neutralizes 2.8 c.c. of the constant-boiling hydrochloric-acid solution). Some distillates, especially those from antimony and bismuth compounds, effervesce with the *zinc* more violently than the solutions in other cases, so in these less than 4 c.c. of acid should be left unneutralized. In order to oxidize the sulphur dioxide in the distillate *strong solution of bromine* is to be added, a few drops at a time, until the colour due to the bromine is permanent even after warming for a minute or two, showing that there is a slight excess. In order to remove this excess, *solution of hydroxylamine hydrochloride* is to be added, a few drops at a time, until the liquid is colourless. The liquid is then to be diluted to 25 c.c. with *water*, and tested as described in Test A. The plug of *plumbized cotton-wool* must be used, as the treatment with bromine does not altogether prevent the evolution of hydrogen sulphide. When the effervescence has ceased a further addition of acid should be made to ensure that all the arsenium is evolved. For the purpose of obtaining a stain for comparison, 3 c.c. of the diluted liquor arsenici hydrochloricus should be submitted to the same process.

METHODS FOR DRUGS SPECIFIED.

The following are the methods to be employed in the cases of particular drugs:—

For *acidum aceticum*, *acidum hydrobromicum dil.*, *acidum lacticum*, *acidum phosphoricum conc.*, *alumen*, *ammonii bromidum*, *ammonii chloridum*, *ammonii phosphas*, *calcii chloridum*, *glycerinum*, *liquor zinci chloridi*, *lithii citras*, *magnesii sulphas*, *phenazonum*, *potassii acetas*, *potassii bromidum*, *potassii citras*, *potassii tartras*, *soda tartarata*, *sodii bromidum*, *sodii sulphas*, *zinci acetas*, *zinci chloridum*, *zinci sulphas*, and *zinci sulphocarbolas*.

Four grms. of the above-named drugs are to be dissolved in nearly 20 c.c. of *water*, and the solution is to be mixed with 5 c.c. or other suitable quantity of *hydrochloric acid*, and diluted to 25 c.c. with *water*, and tested as described in Test A.

For *Potassii Sulphas* and *Sodii Phosphas*.—Four grms. of these drugs are to be dissolved in the smallest convenient quantity of *water*, and the solution is to be mixed with 5 c.c. or other suitable quantity of *hydrochloric acid*, and tested in a small flask as described in Test A.

For *Potassii Iodidum* and *Sodii Iodidum*.—Four grms. of these drugs are to be dissolved in 5 c.c. of *water* and are to be tested by Test A, modified in the following manner: 5 c.c. or other suitable quantity of *hydrochloric acid* are to be mixed with 14 c.c. of *water* in the test-tube. The *zinc* is then to be added and the effervescence is to be allowed to proceed for two minutes, then the above solution of the iodide is to be poured in and the plugs and cap are at once to be put into position. This procedure avoids the liberation of iodine in the liquid; a little iodine appears on the plug but does not materially diminish the stain.

For *Syrupus Glucosi*.—Four grms. of this drug are to be dissolved in 10 c.c. of *water*. In order to oxidize any sulphur dioxide that may be present, 3 c.c. of *strong solution of bromine* are to be added and then 5 c.c. of *hydrochloric acid*, and the mixture is to be warmed for a few minutes, care being taken to stop whilst a distinct amount of free bromine is still present. When cold the free bromine is to be removed by adding a little *solution of hydroxylamine hydrochloride*; 3 c.c. or other suitable quantity of *hydrochloric acid* are to be added, and the liquid is to be diluted to 25 c.c. with *water* and tested as described in Test A. In presence of glucose the stain obtained from 3 c.c. of the diluted liquor *arsenici hydrochloricus* is only about three-fourths of its proper intensity, and for this diminution allowance must be made by means of a comparative experiment made with the *syrupus glucosi* and the *arsenium* solution. The effervescence should be prolonged by a second addition of *hydrochloric acid*.

For *Acidum Boricum* and *Borax*.—Four grms. of these drugs are to be mixed with 8 grms. of *citric acid* and dissolved in 55 c.c. of *water*, and the solution is to be mixed with 5 c.c., or other suitable quantity of *hydrochloric acid*, and tested in a small flask as described in Test A.

For *Acidum Citricum* and *Acidum Tartaricum*.—Twelve grms.

of these drugs are to be dissolved in 40 c.c. of *water*, and the solution is to be mixed with 15 c.c. or other suitable quantity of *hydrochloric acid*, and tested in a small flask as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus similarly treated, thus proving that the drugs contain less than one part of arsenium in one million parts of the drug.

For Acidum Hydrochloricum.—Forty grms., or 34·5 c.c., of this drug are to be placed in a porcelain basin and mixed with 2 c.c. of *strong solution of bromine*. The mixture is to be gently evaporated on a sand-bath, adding small quantities of *strong solution of bromine* from time to time, so that the liquid is always kept orange-colour and smelling of bromine (about 4 c.c. or 5 c.c. of the *strong solution of bromine* will be required in all). The presence of free bromine prevents loss of arsenium during the evaporation. When the volume is reduced to about 15 c.c. the acid is to be partially neutralized with *strong solution of ammonia* (1 c.c. of this neutralizes 2·8 c.c. of hydrochloric acid solution of the constant-boiling strength), so as to leave unneutralized 5 c.c. or other suitable quantity of hydrochloric acid solution of the constant-boiling strength. The excess of bromine is to be removed by adding a little *solution of hydroxylamine hydrochloride*; the liquid is then to be diluted to 25 c.c. with *water*, and tested as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than three-tenths of one part of arsenium in one million parts of the drug.

For Acidum Nitricum.—Forty grms., or 28·2 c.c., of this drug are to be mixed with 2 c.c. of *sulphuric acid*, and with 0·1 gm. of *sodium bicarbonate*, and the liquid is to be evaporated in a porcelain basin on a sand-bath until all the nitric acid is expelled and fumes of strong sulphuric acid are given off. The residual liquid is to be allowed to cool and is then to be mixed with about 15 c.c. of *water*, and then with 3 c.c. or other suitable quantity of *hydrochloric acid*. The mixture is to be diluted to 25 c.c. with *water*, and tested as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than three-tenths of one part of arsenium in one million parts of the drug.

For Acidum Sulphuricum.—Forty grms., or 21·7 c.c., of this drug are to be mixed with 5 c.c. of *nitric acid*, and with 0·1 gm. of *sodium bicarbonate*, and the liquid is to be evaporated in a porcelain basin on a sand-bath until only about 2 c.c. remain. The residual liquid is to be allowed to cool and is then to be mixed with about 15 c.c. of *water*, and then with 3 c.c. or other suitable quantity of *hydrochloric acid*. The mixture is to be diluted to 25 c.c. with *water*, and tested as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than three-tenths of one part of arsenium in one million parts of the drug.

For Liquor Ammonię Fortis.—One hundred and twenty grms., or 135 c.c., of this drug are to be mixed with 0·1 gm. of *sodium bicar-*

bonate, and the solution is to be evaporated to dryness, or nearly to dryness, on a water-bath. The residue, when cold, is to be dissolved with a mixture of 5 c.c. or other suitable quantity of *hydrochloric acid*, and about 20 c.c. of *water*, avoiding heating except for a minute or two. The solution is to be diluted to 25 c.c. with *water*, and tested as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than one-tenth of one part of arsenium in one million parts of the drug.

For ammonii carbonas, calcii carbonas præcipitatus, calcii hydras, calcii phosphas, calx, liquor potassæ, lithii carbonas, magnesia levis, magnesia ponderosa, magnesii carbonas levis, magnesii carbonas ponderosus, potassa caustica, potassii bicarbonas, potassii carbonas, potassii tartras acidus, sodii bicarbonas, sodii carbonas, zinci carbonas, zinci oxidum, and zinci valerianas.

Four grms. of these drugs are to be dissolved in *hydrochloric acid* and *water*, using enough *hydrochloric acid* to acidify and dissolve the 4 grms. of drug taken, and to produce a suitable effervescence with the *zinc*.

Care must be taken not to warm hydrochloric acid and drug together except for a minute or two, and with only a small area of surface, so as to avoid loss of arsenium. If necessary, loss of arsenium can be avoided by mixing a little *strong solution of bromine* with the *hydrochloric acid* and *water*, so as to oxidize the arsenium to arsenic acid, which is not easily volatilized when heated with hydrochloric acid; when solution is effected the excess of bromine is to be removed by the addition of a little *solution of hydroxylamine hydrochloride*.

The solution is to be diluted if necessary and tested in a test-tube or flask as described in Test A.

If a drug contains any iron, it must be tested as described in Test B.

For Cerii Oxalas.—Four grms. of this drug are to be added to a small flask containing a hot mixture of 15 c.c. of *hydrochloric acid*, 10 c.c. of *water*, and 1 c.c. of *strong solution of bromine*. The mixture is to be heated for about a minute, when the cerium oxalate will dissolve, but a precipitate will very soon separate. As soon as solution has occurred the flask is to be removed from the flame and the acid is to be partially neutralized by the addition of about 7.25 c.c. of *strong solution of ammonia*, and the free bromine is to be removed by the addition of a little *strong solution of hydroxylamine hydrochloride*. The mixture is then to be tested in the flask as described in Test A, shaking it occasionally to promote the circulation of the liquid, which is checked by the presence of the precipitate.

For Iodum.—Four grms. of this drug are to be mixed with 0.1 gm. *sodium bicarbonate*, and then with 3 c.c. of *water* and 4 c.c. of *sulphuric acid* in a porcelain basin, and the mixture is to be heated with stirring until all the iodine is driven off. The residue of sulphuric acid is to be diluted with about 15 c.c. of *water*, and then mixed with 2 c.c. or other suitable quantity of *hydrochloric acid*, and then diluted to 25 c.c. with *water*, and tested as described in Test A.

For Liquor Hydrogenii Peroxidi.—Four grms. of this drug are to be mixed with 4 c.c. of *water* and with 2 c.c. of *sulphuric acid*. *Potassium permanganate* is then to be added in small quantities at a time until the hydrogen peroxide is all decomposed and a slight permanent coloration is produced. The solution is to be mixed with 7 c.c. of *water*, and the coloration is to be destroyed by the addition of a little solution of *hydroxylamine hydrochloride*. Three c.c. or other suitable quantity of *hydrochloric acid* are to be added, and the solution is to be diluted to 25 c.c. with *water*, and tested as described in Test A.

For Potassii Chloras.—Six c.c. of *sulphuric acid* are to be mixed with 3 c.c. of *water*, and the mixture is to be heated. Four grms. of this drug are to be added cautiously in small portions at a time to the above liquid whilst hot. When effervescence has ceased the liquid is to be evaporated in a porcelain basin until only about 2 c.c. of *sulphuric acid* are left. The residue is then to be dissolved in about 15 c.c. of *water* and mixed with 2 c.c. or other suitable quantity of *hydrochloric acid* and diluted to 25 c.c. with *water*, and then tested as described in Test A.

For Potassii Nitras.—Four grms. of this drug are to be added to 4 c.c. of *sulphuric acid* in a porcelain basin, and then heated until all the nitric acid is driven off and fumes of *sulphuric acid* escape. The residue is then to be dissolved in about 15 c.c. of *water*, 3 c.c. or other suitable quantity of *hydrochloric acid* are to be added, and the solution is to be diluted to 25 c.c. with *water*, and tested as described in Test A.

For Potassii Permanganas.—Four grms. of this drug are to be added, in small quantities at a time, to 30 c.c. of *hydrochloric acid*. When it has all dissolved 2 c.c. of solution of *hydroxylamine hydrochloride* are to be added in order to decolorize the liquid, and then about 4 c.c. of strong solution of *ammonia* in order partially to neutralize the free *hydrochloric acid*. One c.c. of solution of *hydroxylamine hydrochloride* is then to be added in order to remove the last traces of free chlorine, and the liquid is to be tested in a flask as described in Test A.

For Calcii Hypophosphis and Sodii Hypophosphis.—A mixture of 12 c.c. of *nitric acid* and 12 c.c. of *water* is to be warmed, and 4 grms. of these drugs are to be added in small quantities at a time, so as to prevent the action being too violent. When all is added the liquid is to be evaporated to dryness on a sand-bath and the residue heated, but not strongly, until the nitric acid has been driven off. The residue, when cold, is to be dissolved in 5 c.c. or other suitable quantity of *hydrochloric acid* mixed with *water*, avoiding loss by warming, or using the bromine and *hydroxylamine-hydrochloride* treatment. The solution is then to be diluted to 25 c.c. with *water*, and tested as described in Test A.

For Phosphorus.—Six cgms. of this drug are to be dissolved by heating them cautiously in a flask of about 100 c.c. capacity, having a small funnel placed in its mouth, with a mixture of 5 c.c. of *nitric acid* and 5 c.c. of *water*. The solution is then to be transferred to a porcelain basin, and in order to oxidize any phosphorus acid 5 c.c. of *nitric acid*

are to be added, and the mixture is to be heated until it has concentrated to about half its volume. In order to remove nitric acid 0.1 grm. of *sodium bicarbonate* is then to be added, and 3 c.c. of *sulphuric acid*, and after mixing the liquid is to be evaporated down to about 3 c.c., and then, in order to decompose any nitrosulphonic acid, the residue is to be allowed to cool and mixed with 10 c.c. of *water*. It is then to be evaporated until fumes of strong sulphuric acid escape; when cold the residue is to be diluted with about 10 c.c. of *water* and mixed with 5 c.c. or other suitable quantity of *hydrochloric acid*, and diluted to a volume of 25 c.c. with *water*, and tested as described in Test A. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus thus proving that the drug contains less than 0.02 per cent of arsenium.

For Sulphur Præcipitatum and Sulphur Sublimatum.—Four grms. of these drugs are to be dissolved by heating them in a large flask, having a small funnel placed in its mouth, with 25 c.c. of *fuming nitric acid*, and adding more *fuming nitric acid* when necessary (about 60 or 70 c.c. will be required). When the sulphur has all dissolved 0.1 grm. of *sodium bicarbonate* is to be added, and the liquid is to be evaporated in a porcelain basin on a sand-bath until all nitric acid is expelled and fumes of sulphuric acid are given off; the volume is to be reduced to about 2 c.c., it is then to be diluted with about 15 c.c. of *water* and mixed with 2 c.c. or other suitable quantity of *hydrochloric acid*, and diluted to a volume of 25 c.c. with *water*, and tested as described in Test A. The amount of arsenium in the *fuming nitric acid* used can be determined by the method described for testing acidum nitricum and allowed for. It should be less than one-tenth of one part of arsenium in one million parts of the acid, and the acid should be free from the impurities mentioned in the case of acidum nitricum, especially iron.

For Acidum Salicylicum, Adeps Lanae, Glusidum, Phenacetinum, Sapo Animalis, Sapo Durus, and Sulphonat.—Four grms. of these drugs are to be mixed with 2 grms. of *magnesia* and 2 grms. of *exsiccated sodium carbonate*, and the mixture is to be made into a thin paste by warming it with a small quantity of *water* and stirring. The mixture is then to be dried and ignited in a porcelain basin or in a porcelain crucible until the volatile organic matter is driven off and the residue is greyish-white. The temperature must not approach a white heat. Fifteen c.c. of *water* are to be mixed with 21 c.c. of *hydrochloric acid* and 3 c.c. of *strong solution of bromine*. The bromine is used in order to oxidize any sulphur compounds and to prevent loss of arsenium by the heating of the liquid, which should be cooled by the use of an outer vessel of *water*. The ignited residue is to be added to this mixture in small portions at a time. When solution is effected (some carbonaceous particles will remain undissolved) the excess of bromine is to be removed by adding a little *solution of hydroxylamine hydrochloride*, and the liquid is to be tested in a flask as described in Test A. The plug of *plumbized cotton-wool* must be used, as the treatment with bromine does not altogether prevent the evolution of hydrogen sulphide. For the purpose of obtaining a stain for comparison, 3 c.c. of the diluted

liquor arsenici hydrochloricus should be submitted to the same process.

For cupri sulphas, ferri phosphas, ferri sulphas, gelatinum, plumbi acetat, quininæ hydrochloridum, quininæ hydrochloridum acidum, and quininæ sulphas.

Four grms. of the above drugs are to be tested as described in Test B.

For Antimonii Oxidum and Antimonium Tartaratum.—Four grms. of the above drugs are to be tested as described in Test B, but as the distillate will still contain a little antimony chloride, the condenser is to be washed free from any traces of antimony chloride, and the distillate is to be re-distilled until about three-fourths of it have collected in the receiver, and this distillate is to be treated as directed in Test B. Twenty-three c.c. of *hydrochloric acid* and no water are to be used with the antimonii oxidum, and a mixture of 20 c.c. of *hydrochloric acid* and 3 c.c. of water with the antimonium tartaratum.

For Antimonium Nigrum Purificatum and Antimonium Sulphuratum.—Four cgms. of these drugs are to be heated in a flask of about 100 c.c. capacity, having a small funnel placed in its mouth, with 10 c.c. of *fuming nitric acid*, until all sulphur or black sulphide has been oxidized. A white precipitate will be formed in the liquid, but the absence of free sulphur or of black sulphide can be easily seen. The mixture is then to be transferred to a porcelain basin, and is to be mixed with 0.1 grm. of *sodium bicarbonate* and with 3 c.c. of *sulphuric acid*. All the nitric acid is to be removed by evaporating the mixture down to about 3 c.c., and then, in order to decompose nitrosulphonic acid, mixing the residue when cold with 10 c.c. of water, and evaporating again until fumes of strong sulphuric acid escape. When the residue is cold it is to be transferred to a flask of about 60 c.c. capacity, by means of a mixture of 15 c.c. of *hydrochloric acid* and 7 c.c. of water. Two grms. of *potassium metasulphite* are to be added, and the flask is to be immediately attached to a condenser and treated as described in Test B, but as the distillate will still contain a little antimony chloride, the condenser is to be washed free from any traces of antimony chloride, and the distillate is to be re-distilled until about three-fourths of it have collected in the receiver, and this distillate is to be treated as directed in Test B. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than 0.03 per cent of arsenium.

For Bismuthi Oxidum.—Four grms. of this drug are to be tested as described in Test B, using 20 c.c. of *hydrochloric acid* and 2 c.c. of water; but if the drug contains any nitrate it must be tested in the same manner as bismuthi carbonas.

For Bismuthi Carbonas and Bismuthi Subnitras.—Four grms. of these drugs are to be mixed with 5 c.c. of *nitric acid* in order to oxidize any arsenious compounds to arsenic acid, and then with 8 c.c. of *sulphuric acid*, and the mixture is to be heated in a porcelain basin on a sand-bath until all the nitric acid is expelled and a considerable proportion of the sulphuric acid has been driven off in fumes. When

evaporating off sulphuric acid, in order to avoid loss of arsenium, the latter should be present as arsenic acid and not as arsenious compounds. The residue is to be allowed to cool, and then 6 c.c. of water are to be added. The mixture is again to be allowed to cool, and is then to be transferred to a flask of about 60 c.c. capacity, together with 17 c.c. of *hydrochloric acid*; 4 grms. of *ferrous sulphate* and 2 grms. of *potassium metasulphite* are to be added, and the rest of the test is to be conducted as described in Test B.

For Bismuthi Salicylas and Liquor Bismuthi et Ammonii Citratis.—Four grms. of these drugs are to be mixed with 2 grms. of *magnesia* and 2 grms. of *exsiccated sodium carbonate*, and the mixture is to be made into a thin paste by warming it with a small quantity of water and stirring. The mixture is then to be dried and ignited in a porcelain basin or in a porcelain crucible until the volatile organic matter is driven off and the residue is greyish. Fifteen c.c. of water are to be mixed with 21 c.c. of *hydrochloric acid*, and 3 c.c. of *strong solution of bromine*, in a flask of about 60 c.c. capacity. The bromine is used in order to convert the arsenium into arsenic compounds, and prevent its loss by the heating of the liquid, which should be cooled by the use of an outer vessel of water. The ignited residue is to be added to this mixture in small portions at a time. When solution is effected (some carbonaceous particles will remain undissolved), the flask is to be attached to a condenser as described in Test B, and distilled until about half the volume of the liquid has passed over. This distillate will contain the free bromine and no arsenium; but for greater security it may be tested for arsenium. A fresh receiver is to be placed in position, and 20 c.c. of *hydrochloric acid* are to be added to the residue in the distilling flask, and then 2 grms. of *potassium metasulphite*, and the mixture is to be heated gently for about one hour in order to reduce arsenic compounds to arsenious compounds. It is then to be distilled until about three-fourths of it have passed over, and the distillate is to be treated in the same manner as the distillate described in Test B, but as the volume will exceed 25 c.c., it must be tested in a small flask.

For Ferrum.—Four grms. of this drug are to be dissolved in a mixture of 3 c.c. of *nitric acid* and 3 c.c. of water, and the solution is to be evaporated to dryness in a small porcelain basin, and the residue is to be ignited until the ferric nitrate is converted into ferric oxide. The residue is then to be transferred to a flask of about 60 c.c. capacity, together with 10 c.c. of *hydrochloric acid* and 6.5 c.c. of water, scraping out as much as possible, and treating the remainder with the mixed acid and water, but not warming unless very slightly and only for a minute or two. The flask is to be attached to a condenser, as described in Test B. The mixture is to be warmed until the ferric oxide has all dissolved, and then 4 grms. of *ferrous sulphate* and 2 grms. of *potassium metasulphite* and 7 c.c. of *hydrochloric acid* are to be added, and the rest of the operation is to be conducted as described in Test B. At the end of the distillation the residue in the distilling flask should be tested and some ferrous iron should be found to be present. The stain should be less than that given by 3 c.c. of the

diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than 0.03 per cent of arsenium.

For Ferrum Redactum.—Two decigrammes of this drug are to be heated in a flask having a small funnel placed in its mouth with a mixture of 10 c.c. of *nitric acid* and 10 c.c. of *water*. When the action has ceased, if an insoluble residue is left, it is to be dissolved by adding 3 c.c. of *hydrochloric acid* and continuing the warming. The solution is then to be transferred to a small porcelain basin, and 5 c.c. of *nitric acid* are to be mixed with it, and the liquid is to be evaporated to dryness and ignited until the ferric nitrate is converted into ferric oxide. The ignited residue is then to be treated in the same way as the ignited residue obtained in testing ferrum. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than 60 parts of arsenium in one million parts of the drug.

For Liquor Ferri Acetatis.—Four grms. of this drug are to be put into a flask of about 60 c.c. capacity, together with 4 grms. of *ferrous sulphate*. A mixture of 15 c.c. of *hydrochloric acid* and 2 c.c. of *water* is to be added, and then 2 grms. of *potassium metasulphite*. The flask is then to be attached to a condenser, and the mixture is to be treated as described in Test B. The ferrous sulphate will effect the decomposition of any traces of nitric acid. At the end of the distillation the residue in the distilling flask should be tested, and some ferrous iron should be found to be present.

For Liquor Ferri Perchloridi Fortis.—Twenty-five cgms. of this drug are to be put into a flask of about 60 c.c. capacity, together with 4 grms. of *ferrous sulphate*. A mixture of 15 c.c. of *hydrochloric acid* and 6 c.c. of *water* is to be added, and then 2 grms. of *potassium metasulphite*. The flask is then to be attached to a condenser, and the mixture is to be treated as described in Test B. The ferrous sulphate will effect the decomposition of any traces of nitric acid. At the end of the distillation, the residue in the distilling flask should be tested and some ferrous iron should be found to be present. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than 48 parts of arsenium in one million parts of the drug.

For Liquor Ferri Pernitratis.—One grm. of this drug is to be treated in the same manner as that described above for liquor ferri perchloridi fortis. The stain should be less than that given by 3 c.c. of the diluted liquor arsenici hydrochloricus, thus proving that the drug contains less than 12 parts of arsenium in one million parts of the drug.

The reporters suggest certain alterations in the B.P. monographs so as to provide certain definite limits of arsenium content. The subjoined paragraphs embody their specific recommendations, no attention being paid here to what they say about the text of the monographs.

Antimonium Nigrum Purificatum.—Should be proved to contain less than 0.03 per cent of arsenium by the tests for arsenium.

Antimonium Sulphuratum.—Should be proved to contain less than 0.03 per cent of arsenium by the tests for arsenium.

Ferrum.—Should be proved to contain less than 0.03 per cent of arsenium by the tests for arsenium.

Ferrum Redactum.—To be prepared by reducing ferric hydroxide free from arsenium, heated to dull redness, by a stream of dry hydrogen which has been purified from arsenium compounds. It should be proved to contain less than 60 parts of arsenium in one million parts of the drug by the tests for arsenium.

Glycerinum.—Should yield no characteristic reaction with the tests for arsenium.

Liquor Bismuthi et Ammonii Citratis.—Should yield no characteristic reaction with the tests for arsenium.

Liquor Ferri Perchloridi Fortis.—Should be proved to contain less than 48 parts of arsenium in one million parts of the drug by the tests for arsenium.

Liquor Ferri Pernitratis.—Should be proved to contain less than 12 parts of arsenium in one million parts of the drug by the tests for arsenium.

Phosphorus.—Should be proved to contain less than 0.02 per cent of arsenium by the tests for arsenium.

Sulphur Præcipitatum.—Should yield no characteristic reaction with the tests for arsenium.

Sulphur Sublimatum.—Should yield no characteristic reaction with the tests for arsenium.

THE FOLLOWING DRUGS should also yield no characteristic reaction with the tests for arsenium: acidum boricum, acidum citricum, acidum salicylicum, adeps lanæ, alumen, ammonii bromidum, ammonii carbonas, calcii carbonas præcip., calcii chloridum, calcii hydras, calx, ferri sulphas, gelatinum, glusidum, iodum, liquor hydrogenii peroxidi, magnesia levis, magnesia ponderosa, magnesii carbonas levis, magnesii carbonas pond., magnesii sulphas, phenacetinum, phenazonum, potassii carbonas, potassii chloras, potassii citras, potassii tartras, potassii tartras acidus, quininæ hydrochloridum, quininæ hydrochlor. acid., quininæ sulphas, sapo animalis, sapo durus, soda tartarata, sodii bicarbonas, sodii carbonas, sodii hypophosphis, sodii phosphas, sodii sulphas, sulphonas, syrupus glucosi.

There are so many methods, many merely slight modifications of each other, for the detection and estimation of arsenic in traces, that only a few which are fairly accurate and satisfactory will be described. It is to be noted that Dunstan and Robinson in their prepared standards, have given them in terms of the metal arsenium, which is contrary to the usual practice. In any references in this work, the amount of arsenic, expressed as As_4O_6 is intended unless otherwise indicated.

F. C. J. Bird ("Pharm. Journal," 4, 19, 424) has criticized the above detailed report. He points out that the method of fastening the cap of mercurialized paper is left to the judgment of the operator. He considers that it should be tied tightly over the mouth of the test-tube or flask, so that the evolved gas is obliged to force its way through the pores of the mercurialized paper.

With regard to the intensity of the stain, he points out that it is

diminished by moisture. This should be emphasized, for a given stain, having naturally absorbed moisture by contact with the evolved gas charged with aqueous vapour, will often nearly double its depth of colour on exposure to a temperature 80° – 90° for a minute or two. It would, therefore, appear to be a desirable addition to the directions that the stain from the material under examination and that from the standard arsenical solution, for comparison, should be placed in a water oven for a few minutes, in order to ensure equal conditions and guard against one stain being damper, and, therefore, fainter than the other. Drying in a water oven will also sometimes render evident a stain otherwise indistinguishable.

He further points out—a fact confirmed by the author's experience—that it is necessary to pass the evolved gas through a solution of lead acetate, as cotton-wool soaked in the solution may fail to arrest all the possible H_2S present in a rush of gas. He also recommends placing the stained papers in a little HCl on watch glasses and heating to the boiling-point of the acid, and drying the stains. Any stain due to H_2S would be destroyed, and the resulting brick-red colour is more characteristic and more easily compared with the standard stains. Bird has modified Gutzeit's test with considerable success as outlined below, and in the author's experience, this modified process yields exceedingly accurate results, even with substances so refractory as reduced iron or oxide of iron.

This improved test, in which all the disadvantages and unreliability of the Gutzeit reaction are overcome, consists in evolving hydrogen in a flask from definite quantities of pure zinc and hydrochloric acid at a boiling temperature in presence of a definite amount of the substance to be examined. Any arsenic present is converted into arseniuretted hydrogen, and this, together with the excess of hydrogen and water vapour, is passed through a vertical condenser when the aqueous vapour is condensed and runs back into the flask. The gases are then made to bubble through lead acetate solution (to remove SH_2) and then force themselves through the pores of a small disk of filter paper impregnated with mercuric chloride. Here, if in small proportion, the whole of the arsenic is arrested forming a characteristic lemon-yellow stain, but to guard against any escaping the first disk, a second disk is employed to absorb the last traces. The two disks are then removed and heated in a watch glass with pure HCl , when, if the yellow stain be due to arsenic, it at once assumes a characteristic brick-red hue, differing in this respect from a sulphuretted stain which disappears, a phosphoretted stain which remains yellow, and an anti-moniuretted stain which turns grey and nearly fades. The brick-red paper disk is then treated with hydrochloric acid containing a little bromine, when the stain dissolves and the solution (now containing the arsenic) may be transferred to a small test-tube and a special stannous chloride reagent added, when the brown arsenical coloration is developed either immediately or on standing. In this manner $\frac{1}{100}$ mg. of As_2O_3 can be detected and identified quite easily.

Description of the Apparatus.—A, flask capacity of about 100 c.c., D, bent thistle tube passing through I. R. cork to bottom of A. C,

separator with stopcock, capacity about 50 c.c. B, vertical condenser connected with F by bent glass tube E. *r.r.* rubber joints. F, gas washing bulb containing a 1 in 10 solution of lead acetate. G, special glass nozzle carrying a disk of mercuric chloride paper. *r'r'*, rubber joints. H, second glass nozzle similar to G. J, curved glass open nozzle. I, Bunsen burner.

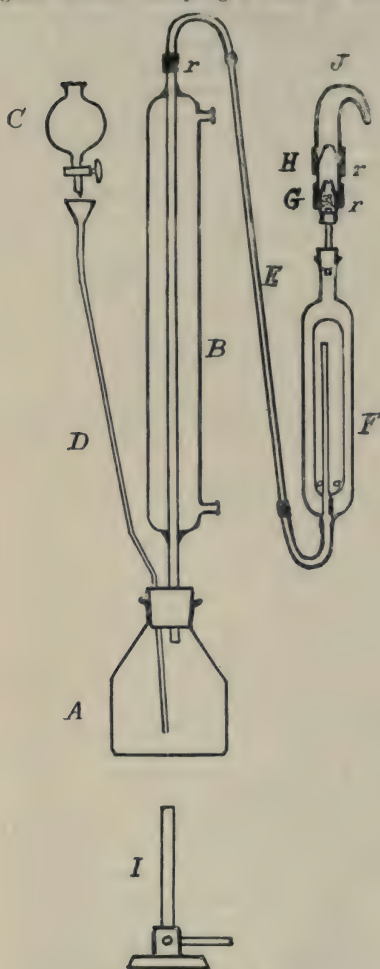


FIG. 58.—Bird's arsenic apparatus.

Directions for use.—In the flask A place 30 c.c. water, four grms. of zinc (distilled, As.-free) and a weighed or measured quantity of the substance to be tested. Introduce into C 15 c.c. of pure hydrochloric acid (As.-free) and heat the contents of the flask A to boiling-point. Then open the stopcock to such an extent that the hydrochloric acid runs out in a steady succession of drops so that about seven minutes are required for the whole to pass into A. The contents of A should be kept in a gentle state of ebullition throughout the experiment. The evolved gas first passes

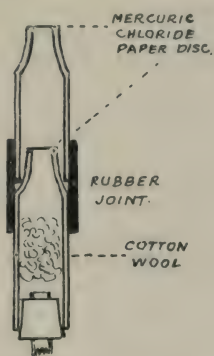


FIG. 59.—Enlarged diagram of exit nozzles in Bird's arsenic apparatus.

through the lead acetate solution F (where SH_2 is removed) and then through the pores of the mercuric chloride paper disks fixed on G and H, which should absorb the whole of the arsenic, finally escaping at J. At the expiration of about fifteen minutes the paper disks

should be detached by touching the edges of the disks with a moistened glass rod, placed in a watch glass, dried for a minute or so in a water oven, and about 3 c.c. pure HCl (*free from Cl*) added, and the whole heated on a thin piece of asbestos millboard until the acid just commences to boil. Any yellow stain due to arsenic now becomes of a deep brick-red, a reaction entirely characteristic of an arsenical stain, all interfering stains (S, P, Sb) behaving quite differently under this treatment. (See F. C. J. Bird, "Analyst," xxvi. 181.) The depth of stain should then be compared with that produced by $\frac{1}{100}$ of a mg. of As_4O_6 , which amount forms a convenient standard for comparison. With regard to the standard, with very pure zinc and HCl $\frac{1}{100}$ mg. is quite definite, but $\frac{1}{50}$ mg. will often be found more convenient.

For ordinary testing the process may terminate here and the further confirmation be omitted.

For further confirmation of the arsenical nature of the stain, the hydrochloric acid is poured away, leaving the paper disks on the watch glass and a second 3 c.c. of pure hydrochloric acid added, warmed gently, and again poured off. (The second washing with acid is for the purpose of removing from the paper excess of mercury salt which would otherwise affect the stannous chloride reaction.)

The disks are now warmed with $\frac{1}{2}$ c.c. hydrochloric acid and the arsenical compound dissolved by the addition of 1 or more drops of bromine hydrochloric acid, avoiding any large excess. The pale yellow liquid is poured off into a small test-tube 3 inches by $\frac{1}{4}$ inch, any residual acid displaced with one or two more drops of hydrochloric acid, an equal volume of 30 per cent stannous chloride solution in HCl added, and the contents of the test tube warmed, when if $\frac{1}{100}$ of a mg. of arsenic be present, the characteristic pink brown coloration of Bettendorff's reaction makes its appearance almost immediately.

Reagents.—Bromine hydrochloric acid. This is strong hydrochloric acid containing sufficient bromine to impart a deep yellow colour.

The stannous chloride reagent is prepared by dissolving 30 grms. of crystallized stannous chloride in 150 c.c. of pure hydrochloric acid and boiling down with a few fragments of metallic tin to 100 c.c. It should remain perfectly colourless when heated with an equal volume of hydrochloric acid and allowed to stand for some time.

Mercuric chloride disks 5 mm. diameter. Filter paper is saturated with a solution of mercuric chloride (1 in 20) and dried; the disks are cut out with a cork borer.

By taking an amount of the substance under examination corresponding to any standard limit of arsenical impurity for that substance, it is possible to see with the minimum expenditure of time and trouble whether the limit has been exceeded or not. In this way with a limit of $\frac{1}{100}$ of a grain per gallon (e.g. beer) 70 c.c. would be taken for the test = 1 part in seven millions. With 7 grms. of material $\frac{1}{100}$ of a mg. of arsenic equals $\frac{1}{100}$ of a grain per lb. With 3.5 grms. $\frac{1}{50}$ of a grain per lb., with 1 gm. 1 part in one hundred thousand, etc.

Cowley and Catford recommend ("Pharm. Journ." **4**, 19, 897) the following process:—

A few inches of fine copper wire, coiled into a spiral are immersed in 10 c.c. of the liquid to be tested, to which one-fifth of its volume of pure HCl has been added. The liquid is contained in a test-tube, which is supported upright in a brine bath. The coil of wire is arranged so that it shall reach from the bottom of the arsenical liquid to above its surface. The test-tube must be immersed in the brine bath so that the liquid it contains shall be below the level of the liquid in the bath; the bath is kept simmering without, however, reaching the boiling-point, for about an hour. The projecting end of the copper is now pressed below the surface of the liquid, and if it (that is, the bright end that has not been previously in the liquid) remains bright after remaining for another fifteen minutes, the arsenic will be all removed from the liquid, and the wire may be removed to a small dish, rinsed without touching it with the fingers, and the deposit then dissolved off by 1 c.c. of bromine water containing a little hydrobromic acid. The clean wire is lifted out, rinsed with water, and if thought necessary may be returned to the acid liquid to make sure that all the arsenic has been dissolved from it. The bromine solution now contains the arsenic as arsenious acid. To it 1 c.c. of solution of potash is added, and the liquid is boiled until the light green copper compounds are broken up. The arsenic in the neutralized filtrate is reduced completely to arsenite and titrated with $\frac{N}{100}$, or other suitably weak

solution of iodine. A solution of iodine of convenient strength is made by diluting 10 c.c. of $\frac{N}{10}$ solution to about 150 c.c., and comparing it with a standard arsenical solution.

For a burette a pipette graduated in hundredths of a cubic centimetre is used. To control the flow, a piece of rubber tubing is slipped on the upper end and compressed by a screw clamp. One-hundredth part of a c.c. of the iodine solution gives a blue colour, with starch, in a volume of liquid not exceeding 10 c.c.

For the detection of arsenic in such liquids as beer (which is conveniently described here) Hehner recommends the following process, which is based on the Marsh-Berzelius method:—

The hydrogen is obtained from zinc and hydrochloric acid. It is important that the zinc should be arsenic free, and sensitive to the presence of arsenic under the conditions of the test. The fact pointed out by Dyer, that certain forms of zinc do not yield a good mirror is confirmed, Hehner stating that this is the case with the metal cast in rods. Granulated zinc should be used. The hydrochloric acid should be subjected to vigorous boiling to drive off every trace of arsenic. About 10 grms. of zinc are then introduced into a 250 c.c. flask, fitted with a two-hole rubber cork. Through one hole passes a tap funnel, through the other, the exit tube, connected with a tube holding a roll of dry lead acetate paper, then a plug of cotton-wool, then granulated calcium chloride, about 3 inches in length, and then another plug of wool. To this is attached the hard-glass reducing tube, quill-

size, drawn out in the middle to a thickness corresponding to a standard wire gauge No. 13 (0.092 inch) at the place where the arsenical mirror is to make its appearance. Five c.c. of water are run into the flask, followed by 10 c.c. of the pure HCl. The issuing hydrogen is then ignited, a Bunsen flame applied to the reducing tube, and the time noted. The Bunsen is removed in fifteen minutes, when no arsenical mirror should be apparent. Having thus assured the absence of arsenical impurity in the reagents and apparatus, 10 c.c. of beer are slowly dropped in, care being taken to introduce no air, and the Bunsen flame reapplied to the reducing tube. If frothing occurs a little strong alcohol may be used, but if possible this should be avoided. The test should be continued for fifteen minutes. The mirror-bearing tube is now disconnected, hydrogen removed by suction, and the narrow parts fused up on both sides of the mirror. On gently drawing this closed tube through a flame until the mirror disappears, the arsenic therein is oxidized by the contained air, and, on cooling, glistening crystals of As_4O_6 will be obtained, which are evident to the naked eye with even so little as one or two-thousandths of a mg. Selenium and tellurium do not, in Hehner's opinion, interfere with the production of the mirror. For quantitative determination, a series of standard mirrors should be prepared by this method, with arsenical solutions of known strength which should not, at the maximum, contain more than 0.01 mg. These standard mirrors are fused off, mounted on white card, and kept in the dark, for comparison with those obtained in ordinary tests. With sulphuric acid, a preliminary test with 10 grms. diluted with water should be made. If the mirror be too strong, a fresh experiment with a less quantity should be performed. With glucose and sugar, 10 grms. is a convenient quantity to work with. In the case of malt, 10 grms. should be washed with dilute HCl at first cold, then warmed, three or four times and the test made on the extract.

Allen ("Chem. News," LXXXII. 305) prefers the following modification of the Reinsch test. Pure hydrochloric acid from which the first 10 per cent has been removed by vigorous boiling, so as to ensure freedom from arsenic, is employed. One hundred c.c. of beer are used for the analysis, which is first boiled for a few minutes with a little HCl and bromine water. To ensure the reduction of *arsenic* to *arsenious* oxide, a little cuprous chloride in hydrochloric acid solution is then added. About 1 c.c. of copper foil is now introduced and the whole boiled for 30 minutes, water being added to keep the volume approximately constant. If the copper has been stained, it is dried in the water oven, cut into strips and heated in a long narrow tube, when characteristic crystals of arsenious oxide acid are visible. In doubtful cases, it is best to repeat the whole test several times, and submit the combined deposits to Marsh's test as described by Hehner above.

For the quantitative determination of arsenic Allen ("J. S. C. I." xx. 197) modifies the above process.

One litre or 500 c.c. of the beer, according to the qualitative indication of arsenic, is evaporated down to 200 c.c. in a basin, about 20 c.c. bromine water and 20 c.c. hydrochloric acid added, and the excess of bromine boiled off, the volume of the liquid being kept at about 200

c.c. A few drops of a freshly prepared solution of cuprous chloride in hydrochloric acid, and three or four pieces of pure copper foil are then added, and the boiling continued for half an hour. The pieces of copper are removed and replaced by fresh ones till no darkening takes place. They are treated in a beaker with hydrochloric acid and crystals of potassium chlorate, taking care to have excess of the latter, till the arsenic is removed. The solution is warmed till free from oxides of chlorine, and transferred to a distilling flask. An alternative method, due to Clark and Jones, may be used. This is to cover the copper with water in a beaker, add 10 c.c. of 5 per cent caustic soda and 10 drops of solution of hydrogen peroxide, and allow to stand, in the cold, till the arsenic is dissolved. A few drops of cuprous chloride solution and about 15 c.c. hydrochloric acid are added and the liquid distilled into water till the residue in the flask measures about 15 c.c. The distillation is repeated with 20 c.c. fuming hydrochloric acid, the combined distillates rendered alkaline with ammonia, and then slightly acidified with hydrochloric acid, keeping cool by immersion in water. It is then neutralized with sodium bicarbonate, a slight excess of sodium bicarbonate added, and titrated with $\frac{N}{200}$ iodine solution (using starch as an indicator), 1 c.c. of which represents 0.0002475 grm. As_4O_6 .

A blank determination should be made on the reagents employed, the amount of iodine solution required being deducted. The hydrochloric acid, copper, cuprous chloride, caustic soda, and hydrogen peroxide are all liable to contain traces of arsenic.

Thorpe ("Journ. Chem. Soc." **83**, 974) has recommended a process which depends on the electrolytic formation of hydrogen, instead of the use of zinc and acid. In the author's opinion it is too complicated for general use and does not give any better results than Hehner's modification of the Marsh-Berzelius test.

LEAD.

The committee of reference in pharmacy have recommended a quantitative test for the determination of lead, to the British Pharmacopœia committee, which is based on the colorimetric test of Warington.

The following are the contents of this report:—

QUANTITATIVE COLORIMETRIC LEAD TEST.

Apparatus.

Note.—All glass apparatus used should be lead-free.

Nessler Glasses.—These should be thin and of lead-free glass. They should be about 25 mm. in diameter, and about 100 mm. in height to the 50 c.c. mark.

Solutions.

Strong Lead Solution.—Dissolve 0.16 grm. of pure re-crystallized lead nitrate in water, adding 50 c.c. of strong nitric acid, and dilute

with water to 100 c.c. This solution is of such strength that 1 c.c. = 0.001 grm. Pb. and forms a permanent stock solution.

Dilute Lead Solution.—Dilute 1 c.c. of the strong lead solution, measured from a burette, with water so that the resulting solution measures 100 c.c. This solution is of such strength that 1 c.c. = 0.00001 grm. Pb. It is the solution actually used in the tests and should be freshly prepared.

Potassium Cyanide Solution.—Dissolve 10 grms. of potassium cyanide (98 per cent) in water, add 2 c.c. of solution of hydrogen peroxide and make up to 100 c.c.

Note.—This solution, after being allowed to stand, should be tested under the conditions of the quantitative colorimetric test to see that it gives no colour with the dilute lead solution.

Sodium Sulphide Solution.—Dissolve 10 grms. of crystallized sodium sulphide in water and make up to 100 c.c.

Mode of Testing (General).

Two solutions in hot water of the substance under examination are made:—

- (1) The primary solution containing 12 grms. of the substance.
- (2) The dilute solution containing 2 grms. of the substance.

Each solution is filtered (if necessary), made alkaline with ammonia, and treated with 1 c.c. of the potassium cyanide solution.

If the colour of the two solutions differ much, this may be rectified by the cautious addition of a highly diluted solution of burnt sugar.

Then, by the method of trial and error (well known in water analysis as "Nesslerizing") is determined the quantity of dilute lead solution which must be added to the dilute solution, in order that there may be equal colorations produced upon the addition of two drops of sodium sulphide solution to both the primary and dilute solutions, after dilution to the 50 c.c. mark. In these circumstances, each c.c. of lead solution required represents 1 part per million of lead in the substance examined.

The colorations may be viewed by light reflected from a white tile through the Nessler glasses inclined at an angle to the observer.

Note.—In some cases, 7 or 4 grms. only are used in the primary solution. In these cases each c.c. of dilute lead solution required will represent 2 or 5 parts per million of lead respectively.

This report has been criticized somewhat severely by Harvey and Wilkie ("Chemist and Druggist," 1909 ii. 92) with whose remarks the author, in the main, agrees. They state that in most cases inconveniently large quantities of substance are used, the natural limitations imposed by solubility not being taken into consideration. This is objectionable, as one must either work with hot solutions or permit a certain amount of crystallization to occur. Pure metallic lead forms a better standard than the nitrate, and in preparing the strong lead solution it is highly necessary to cool before finally adjusting to volume. As alkaline hydrogen peroxide under certain conditions oxidizes lead sulphide to the sulphate in the cold, the potassium-cyanide solution should be tested to ensure that it exercises no apparent solvent action

on lead sulphide. The committee has also failed to direct that solutions should be cooled to laboratory temperature before adding the sodium sulphide, failing which consistent results cannot be obtained. They do not approve the viewing of the tests at an angle inclined to the observer. It is much better to stand them on a good white surface and view them from above.

The recommendation to filter the aqueous solution is obviously bad as, in the case of cream of tartar for example, lead often occurs as minute particles of metallic lead, which would thus be filtered off and ignored. Since iron and copper frequently occur with lead as impurities, these have to be reckoned with and as potassium cyanide causes a yellow colour to appear with iron salts, it renders the process unworkable when more than the faintest traces of iron are present. The use of a colouring matter such as burnt sugar is also to be deprecated.

The addition of tartaric acid prevents the interference of iron with the test. Teed ("Analyst," xvii. 142) pointed this out, and recommends the following details for carrying out the test:—

A measured quantity of the liquid is mixed in a cylinder or white basin with a few c.c. of ammonia, a few drops of solution of potassium cyanide, and then with a drop of ammonium sulphide. A small quantity of pure tartaric acid is added unless it be already present. The presence of lead will be indicated by the dark coloration produced, and its quantity can be estimated by imitating the coloration with known quantities of lead precipitated under the same conditions. Iron does not interfere with the test, as it is kept in solution by the tartaric acid, and is then converted by the potassium cyanide into a ferro- or ferricyanide, which is not affected by ammonium sulphide. Copper does not interfere with the test, as copper sulphide is soluble in potassium cyanide.

As a very delicate test for the detection of lead in sulphuric acid, Teed proposes the addition to the strong acid of a drop of hydrochloric acid or of a small crystal of common salt. Chloride of lead is thus precipitated and recognized by a peculiar pearly opalescence of the liquid.

The colorimetric process is due in the first instance to Warington. Bennet ("Chemist and Druggist," 64, 633) gives the following details for the determination of lead in nitric and tartaric acids and cream of tartar. In other cases, when these details are applicable, tartaric acid, as recommended by Teed, should be added to prevent the interference of iron salts.

Ten grms. are dissolved in 15 c.c. of distilled water, 25 c.c. of solution of ammonia (10 per cent) added (for cream of tartar 10 c.c. is sufficient) and made up to 50 c.c. One drop of solution of sodium sulphide (10 per cent) is added, and the coloration produced is matched in Nessler glasses by adding from a burette a standard solution of lead acetate (containing 0.0001 grm. of lead in 1 c.c.) to 50 c.c. of distilled water containing a drop of sodium sulphide solution. Each tenth part of 1 c.c. will then represent 1 part of lead per million.

If iron be present, the addition of 1 c.c. of a 10 per cent solution

of potassium cyanide is necessary, copper also being eliminated since copper sulphide is soluble in potassium cyanide. A yellow coloration is often caused by the addition of the cyanide, but this gradually disappears on warming. If only slight, it may be matched before adding the sodium sulphide, and the amount of standard lead solution so used deducted from the total quantity required. It is essential that the solution should be distinctly alkaline, or the full colour is not developed.

Sodium sulphide is much preferable to either sulphuretted hydrogen or ammonium sulphide, as no turbidity or coloration is produced in the absence of metals, while its comparative freedom from odour is also a distinct advantage.

According to Carles, one of the leading French experts on the wine and tartar industries, lead exists in cream of tartar, not as tartrate but as sulphate dissolved by the bitartrate. To detect and estimate it in cream of tartar Carles uses 10 grms. of the sample. It should be finely powdered, carbonized in a porcelain capsule, extracted with hot water, and filtered through a small folded paper. The insoluble sulphate and carbonate of lead remain with the carbon on the filter, which is washed to get rid of potassium carbonate. It is then treated with dilute nitric acid and filtered.

The filtrate is rendered alkaline with excess of ammonia, and the solution containing any copper present is filtered off, the precipitate is dissolved in hot dilute HCl and the lead precipitated as sulphide and weighed. With minute quantities of lead, however, this gravimetric process does not give very useful results.

If iron be absent, the amount of copper present can be *approximately* determined by (1) matching the coloration of a dilute acetic acid solution of the substance with a few drops of potassium ferrocyanide solution, against standard amounts of a solution of pure copper sulphate containing 0.1 mg. of Cu per c.c.

(2) By determining the lead by the sodium sulphide coloration process, and at the same time preparing a solution under identical conditions, but without the addition of potassium cyanide. A solution of copper sulphate (equivalent to 0.1 mg. Cu per c.c.) is then added to the tube containing the standard amount of lead, until the colour is matched, which then gives approximately the amount of copper also.

A satisfactory method of determining the copper in the presence of lead is an electrolytic process, but if only slight traces are present, the amounts obtained are too small to weigh accurately. The details of the experiment must be carefully observed, since whilst lead separates normally as an oxide at the anode, some of it sometimes separates simultaneously in the metallic form at the kathode. Sulphuric acid, as recommended by Hill ("Chemist and Druggist," 23 May, 1908), is not so suitable as nitric acid. It is necessary to use at least 100 grms. of most chemicals, dissolved in about 200 c.c. to 250 c.c. of a mixture of 9 parts of water and 4 of concentrated nitric acid.

A current of 1.0 to 1.5 ampères should be maintained, with an electromotive force of about 1.4 volts. Under these conditions, all

the lead will be separated as peroxide of lead at the anode; this electrode is removed, washed with distilled water, dried at 180° to 200° and weighed. The increase in weight may be calculated as PbO_2 , and if multiplied by 0.866 gives the amount of metallic lead. A fresh electrode is put in, and the current allowed to continue for about 4 hours when the copper is entirely deposited, and is weighed on the kathode. It is convenient to use a "cone" or "jacket" electrode as the anode, the kathode being smaller. After replacing the cone by a fresh one, the current should be reversed and the copper originally deposited on the smaller surface, is dissolved again by the electrolyte and it is all deposited on the cone which is now acting as kathode.

Numerous experiments have convinced the author that the following method is the most accurate available. It is the combination of the principle, first suggested (so far as the author can trace) by C. Hill, that while the trustworthiness of these colorimetric tests is dependent upon the comparison being made between two solutions of the same substance, it is independent of the concentration of those solutions within wide limits.

With the necessary corrections for the presence of iron suggested by either Teed or Harvey and Wilkie, it is substantially that of the report of the Reference Committee detailed above, with the slight modifications necessary when iron is present, and is that given by Harvey and Wilkie in the criticism already mentioned.

Mode of Testing (General).—Two solutions of the substance under examination are made in water—(1) The primary solution, containing 5 grms. of the substance; (2) the dilute solution, containing 2.5 grms. of the substance and 5 c.c. of the dilute lead solution. The volume of each solution should be about 40 c.c. Four drops of acetic acid (33 per cent) are added and then 1 c.c. of the potassium-cyanide solution. After well mixing, excess of .880 ammonia is added. The volume of each is adjusted to 50 c.c. at laboratory temperature, and two to three drops of sodium-sulphide solution are added. Under these conditions the coloration developed in the primary solution should not be darker than that of the comparison solution, thus ensuring that the lead present does not exceed twenty parts per million.

NOTES.—In a few cases 7.5 grms. are used in the primary solution, the comparison solution as usual containing 2.5 grms. and 5 c.c. of the dilute lead solution. If the colour in the primary solution be darker it must be matched and the amount of lead calculated.

Should any insoluble matter be present, the solutions should be boiled if necessary with more acetic acid, correspondingly more ammonia being subsequently used.

The primary and secondary solutions prior to the addition of sodium sulphide must be colourless; if this is not the case special treatment must be given (cf. *infra*); in addition they must in all cases be cooled to laboratory temperature.

In all cases where the acid solutions, treated with potassium cyanide, and then by ammonia, give a colourless solution, or one so faintly yellow as to be almost inappreciable, the interference of iron need not be feared. But if this be not the case then, if the colour be due to ferric iron, it will disappear on the addition of a little more ammonia and

gently boiling. If this is not the case, a little tartaric acid may be added to a fresh preparation, and if the solution still remains coloured after treatment with potassium cyanide and ammonia, then Harvey and Wilkie's more drastic treatment should be employed. This is as follows:—

After solution in water, four drops of hydrochloric acid, specific gravity 1.16, is added, then 1 c.c. of a saturated aqueous solution of sodium sulphite ($\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$), and the solution heated gently until the colour due to ferric iron suddenly bleaches. To the colourless solution is added a mixture of 1 c.c. 10 per cent potassium-cyanide solution and 2 c.c. .880 ammonia. The whole is again heated and gently boiled, if necessary, until quite colourless; then it is cooled, adjusted to 50 c.c. and treated with sulphide in the usual manner.

SACCHARIN.

Saccharin, ortho-benzoic-sulphinide $\text{C}_6\text{H}_4 \begin{matrix} \diagup \text{CO} \\ \diagdown \text{SO}_2 \end{matrix} \text{NH}$, is official in the British Pharmacopœia under the name *Glusidum*.

The official requirements for this substance are as follows: It is soluble in 400 parts of cold water, in 24 parts of boiling water and in 25 parts of 90 per cent alcohol: only slightly soluble in ether and chloroform. Readily soluble in alkalis. When a warm solution of Na_2CO_3 is neutralized with saccharin and the liquid evaporated to dryness, 100 parts yield about 113 parts of "soluble saccharin". Neither saccharin nor soluble saccharin is blackened by H_2SO_4 even when gently warmed. On evaporating with solution of KOH and gently fusing for a few minutes, the residue when cooled and dissolved in water and slightly acidulated with HCl , gives a purplish colour with ferric chloride solution. 0.5 gm. in 80 c.c. of warm water set aside for twelve hours deposits tabular crystals which melt at 218.8° to 220° , and should not, even when briskly shaken, deposit crystals melting at a higher temperature (absence of sulphamido-benzoic acid).

Pure saccharin is a white crystalline powder melting at 220° (according to Allen at 224° , but this is not correct). It is soluble in 50 parts of amyl acetate and in 20 parts of ethyl acetate.

Commercial Saccharin.—In commerce, saccharin occurs in two forms, (1) "550" which is intended to be 550 times sweeter than sugar and which corresponds to the B. P. *glusidum*, and (2) "330" an impure form, stated to be 330 times as sweet as sugar. Each has its corresponding soluble variety.

Mineral adulterants, which may be present, are at once indicated by the ash, which should be infinitesimal in the case of saccharin.

Sugars can be detected by exhausting the solid substance repeatedly with a mixture of equal volumes of ether and petroleum ether. 0.5 gm. should be extracted three or four times by well shaking with successive quantities of 50 c.c. of the solvent. If the undissolved residue contains sugar it will have a sweet taste, and this may be determined by inversion and titration with Fehling's solution, which is not reduced by saccharin.

When unmixed with other substances saccharin may be approximately determined by titration with decinormal alkali, 1 c.c. = 0.0183 grm. of saccharin.

Sulphamido-benzoic acid is an impurity specially recognized in the Pharmacopœia. This acid melts at 281° to 282° and may be detected by shaking 1 grm. with 70 c.c. of ether for five minutes, and filtering and collecting the undissolved residue. As saccharin is far more soluble in ether than the sulphamido-benzoic acid, the latter, if present, accumulates in the undissolved portion, and its melting-point will be above that of saccharin. If the residue melts at above 224° , the presence of this impurity is almost certain.

The Determination of Saccharin in Commercial Samples.—The process of Remsen and Burton gives accurate results ("Amer. Chem. Journ." 1889, 403). Two grms. are boiled under a condenser for an hour with 100 c.c. of dilute HCl. The liquid is evaporated to 15 c.c. when sulphamido-benzoic acid will separate if present. After four hours standing they are collected on a tared filter, washed with a little cold water and weighed. The solution and washings now contain acid ammonium sulphobenzoate, which results from the decomposition of the saccharin in the sample, and any acid potassium ortho-sulphobenzoate which may have been present in the sample. The liquid is evaporated to dryness and the residue weighed. The potassium is then converted into sulphate and weighed. From the weight of the potassium sulphate, the amount of acid potassium sulphobenzoate present can be calculated (its formula is $\text{COOH} \cdot \text{C}_6\text{H}_4(\text{SO}_3\text{K})$). The dry residue minus this gives the weight of acid ammonium sulphobenzoate from which the amount of true saccharin is calculated, by multiplying by 0.835.

Reid ("Amer. Chem. Journ." xxi. [6], 461) has devised a useful and fairly accurate process of assay for commercial saccharin, based on the fact that it is converted into the acid ammonium salt of *o*-sulphobenzoic acid by boiling with hydrochloric acid, whilst *p*-sulphamido-benzoic acid is unaffected.

The process is conducted as follows: 0.650 grm. of "saccharin" is weighed out into a 100 c.c. flask, and 50 c.c. of dilute hydrochloric acid are added (120 c.c. pure concentrated HCl in 1 litre). The flask is fitted with a cork, through which a glass tube passes, 8 mm. wide and 45 cm. long. After two hours' gentle boiling on a sand-bath, the stopper is removed, and the solution allowed to evaporate to about 10 c.c. After diluting, the contents are washed out into an ordinary distilling flask. Twenty c.c. of a caustic soda solution (equal to 10 grms. of NaOH) are added, the ammonia is distilled off into standard acid, and the excess titrated back with KOH, cochineal being the indicator. To cause the caustic soda solutions to boil evenly, steam is passed into the distilling flask, from a flask containing water acidulated with H_2SO_4 .

For alternative processes, Hefelmann ("Pharm. Central" **36**, 228) and Proctor ("Journ. Chem. Soc." 1905, 242) may be consulted.

The Detection of Saccharin in Beverages, etc.—Saccharin may be extracted from foods and estimated by the following method. If a liquid, 50 c.c. are taken; if a solid, a suitable aqueous extract is made by warm alcohol and then diluted with an equal

volume of water. The liquid is concentrated to about 25 c.c. and the alcohol driven off, a little hydrochloric acid added, and the liquid is extracted twice with 25 c.c. of amyl acetate. This solvent dissolves but little colouring matter. The amyl acetate solution is evaporated to dryness, and the residue dissolved in a little bicarbonate of potassium solution. A few drops of lead acetate solution are added, the liquid filtered and the excess of lead removed by H_2S , and the liquid again filtered. It is then extracted twice with ethyl acetate, after being rendered acid with a trace of hydrochloric acid, and the residue dried and tested by taste and other means. The best method for a quantitative determination is to remove as much extraneous matter as possible from the solution containing the saccharin, by means of lead acetate, and then extracting twice with 50 c.c. of a mixture of equal volumes of ether and petroleum ether. The residue obtained on eva-

poration is titrated with $\frac{N}{100}$ sodium hydroxide solution, and almost theoretical results are obtained.

Bianchi and Di Nola ("Boll. Chim. Farm." 1908, **47**, 599) give the following method for detecting saccharin in beverages and foods. The liquid, or in case of a solid a suitable alcoholic extract, diluted with water, is evaporated to free it from alcohol, heated to boiling, and acidified with about 20 drops of acetic acid per 100 c.c. The liquid is cooled and then mixed with 10 c.c. of a 20 per cent lead acetate solution. After half an hour, the excess of lead is removed by a solution containing 10 per cent each of sodium sulphate and phosphate. The filtered liquid is concentrated to 70 c.c., acidified with 6 c.c. to 8 c.c. of 25 per cent sulphuric acid and extracted with its own volume of a mixture of equal quantities of ether and petroleum ether. The residue from this extraction is tested. (1) By tasting, (2) by fusing with potash at 270° , adding a few drops of HCl , then ferric chloride, when a violet colour results, (3) also for salicylic acid which would be present in this residue if present in the beverage, by direct testing with ferric chloride when a violet colour results. If salicylic acid be found the potash fusion test will not, of course, be relied on, until the salicylic acid is removed. In this case the residue should be mixed with dilute HCl , and bromine water added. The precipitated bromine derivative of salicylic acid is filtered off, and the filtrate is rendered alkaline with KOH , and again dried, and the residue, now free from salicylic acid, tested as above.

Allen's method is especially adapted to the detection of saccharin in malt liquors, in the analysis of which bitter substances may interfere with the characteristic taste of the saccharin separated. It is claimed that minute quantities can be detected by this method. The liquid to be tested is evaporated to a small bulk, acidified with a little phosphoric acid, and extracted with ether. The residue from the ether is rendered alkaline with sodium hydroxide, ignited, and the ash tested for sulphate. The presence of sulphate is regarded by Allen as conclusive of the presence of saccharin. The process is facilitated if the liquid is first treated with a little lead acetate solution and filtered. Excess of lead—if not too great—is immaterial.

Kastle ("Jour. Chem. Soc." 1905, 503) gives the following delicate reaction which will detect as little as one quarter of a mg. of saccharin. A small quantity of the solid residue, e.g. the residue from an extraction, is mixed with a minute amount of a mixture of 5 c.c. of phenol and 3 c.c. of sulphuric acid, and heated to 160° to 170° for five minutes. The mass is dissolved in cold water and a little NaOH added. A purple or rose-red will result if saccharin be present.

Truchon gives the following method, which is used in the municipal laboratory of Paris. At least 200 c.c. of liquid, after acidifying with phosphoric acid, are extracted three times with 35 c.c. to 40 c.c. of a mixture of equal parts of ether and petroleum spirit. The extract is washed with water, evaporated in a platinum dish, 5 to 6 drops of a solution of pure caustic soda are added, and the mass is carefully brought to quiet fusion over a small Bunsen flame. The end of the reaction is indicated by the disappearance of the small gas bubbles. The mass is extracted with distilled water, the solution acidified with sulphuric acid, extracted twice in succession with 30 c.c. of petroleum spirit, the petroleum separated, evaporated in a porcelain dish, and a drop of a very dilute (1:10,000) solution of iron chloride added to the residue. If saccharin was originally present, the well-known violet coloration is produced by the salicylic acid formed from the saccharin.

Tertoni ("Zeit. Untersuch. Nahr. Genuss." 1909, **12**, 577) has devised processes for the identification of saccharin in the presence of benzoic, salicylic, and citric acids, etc., which separate with the saccharin. The substance is extracted as described in the above processes, by a mixture of ether and petroleum ether, and the residue obtained by evaporation, tested as follows:—

In the Presence of Benzoic Acid.—Weigh the residue so obtained, in a tared porcelain capsule, and heat in an oven the temperature of which is 110° to 115° C., until the weight becomes stable. The saccharin will remain unchanged whilst the benzoic acid present will be partly volatilized. To get rid of the benzoic acid entirely dissolve the residue in alcohol. The saccharin may be precipitated by adding silver nitrate solution in small quantities. Collect the precipitate on a filter, wash with alcohol, dry at 100° C. then weigh. Its formula is $C_7H_4SO_3Na$, and 37.24 per cent of it is silver. Benzoic acid cannot be precipitated in this way.

In the Presence of Salicylic Acid.—Precipitate the salicylic acid with bromine. Remove the tetra-bromophenol by filtration and then extract the saccharin by petroleum ether and ether.

In the Presence of Fats, Fruit Essences, etc.—If there are fatty substances as well as the saccharin in the residue, the latter can be estimated by using a mixture of sodium carbonate and potassium nitrate to fuse with the residue, then precipitating the resulting sulphuric acid in the usual manner. Heat the fatty residue with hydrochloric acid (sp. gr. 1.1) to a temperature of 120° to 130° C. in an autoclave. The nitrogen of the saccharin is converted into ammonia, and the saccharin can be estimated by rendering the liquid alkaline and distilling the ammonia into normal acid. There must, of

course, be no sulphur and nitrogen compounds except the saccharin in the ethereal extract.

Jorgensen's process is a most useful one for the detection of saccharin where easily-oxidizable interfering substances are present.

Evaporate 500 c.c. of beer on a water bath until it is the consistency of a syrup, then mix the residue with a quantity of 96 per cent alcohol and stir well. Decant the alcoholic solution, evaporate the residue to dryness, then moisten the solid residue with water and again extract with alcohol. The united extracts should be evaporated until all the alcohol has been expelled. Add a few drops of sulphuric acid to the aqueous syrup, filter, and shake out the filtrate with several successive portions of ether. Evaporate the ethereal solution, take up the residue with a little water, add a small quantity of dilute sulphuric acid and saturated potassium permanganate solution, drop by drop, until there is a permanent pink coloration; to remove excess of permanganate add saturated oxalic acid solution, avoiding an excess of oxalic acid. After filtering, extract the colourless solution with a mixture of ether and petroleum spirit.

If the beer contains saccharin, the crystalline residue obtained by evaporating the ethereal extract, will have an extremely sweet taste. The treatment with permanganate will have removed any salicylic acid present in the beer, and the saccharin obtained may be further identified by converting it into salicylic acid and applying the usual tests for the latter substance.

SALICYLIC ACID.

Salicylic acid $C_6H_4.OH.CO_2H$ (ortho-hydroxy-benzoic acid) is an acid which occurs in nature, generally as its methyl ester, in numerous plants; but the greater part of the commercial article is prepared artificially by heating potassium phenate with CO_2 , or by a similar process.

It is official in the Pharmacopœia, which requires it to have the following characters. Soluble in about 500 parts of water, in 3 of alcohol, in 2 of ether and in 200 of glycerin. A 1 per cent solution in alkalis or in certain saline solutions yields a yellowish precipitate with uranium nitrate solution. Melting-point 156° to 157° . Volatile below 200° without decomposition. Solution of ferric chloride gives with an aqueous solution a violet colour. If shaken with water, the water filtered and evaporated, the residue is white without a buff-coloured fringe. It dissolves in cold H_2SO_4 imparting no colour to it in fifteen minutes. If 1 grm. be dissolved in solution of Na_2CO_3 and the liquid shaken with ether, and the ether allowed to evaporate spontaneously, the residue, if any, should not smell of phenol.

Salicylic acid occurs in commerce in four varieties (1) natural, that is made from a naturally occurring methyl salicylate, such as oil of sweet birch, (2) physiologically pure, that is, freed from traces of impurities which *may* have a bad effect therapeutically, (3) crystal, and (4) powder, which may or may not contain traces of such impurities. Apart from its use in medicine, it acts as a very powerful preservative,

and as such is often added to foods and wines, so that its detection is a matter of considerable importance.

Pure salicylic acid melts at 156.7° and has a specific gravity 1.483 at 4° . Traces of impurities such as para-hydroxy-benzoic acid and hydroxy-phthalic acid lower the melting-point appreciably.

This drug is rarely adulterated, but traces of impurities must be guarded against, although the ordinary grades are now usually sufficiently pure for use in medicine.

According to Kolbe the absolute whiteness of the residue obtained on evaporating a solution of 0.5 grm. in 5 c.c. of alcohol, is important. If a coloured deposit is obtained the sample should be rejected.

Phenol should be absent, and the following test (due to Muter) may be applied in addition to that given in the Pharmacopœia:—

Boil 1 grm. in 15 c.c. of water, cool, pour off the solution and add to it 1 drop of a saturated solution of KHCO_3 , 1 drop of aniline and 5 drops of a solution of calcium hypochlorite. In the presence of phenol a deep blue colour will be produced.

The only natural impurity found to-day in salicylic acid made from phenol is para-cresotic acid, which may occur to the extent of 3 per cent in badly made specimens; whilst twenty years ago as much as 15 to 20 per cent of this and similar impurities was common. Fischer has devised the following method for the detection of para-cresotic acid. CaCO_3 (1 to 2 grms.) is boiled with 15 c.c. of water and 3 grms. of the acid. The flask is kept well shaken over a flame until the volume is reduced to 5 c.c. The liquid is cooled and the crystals separated by filtration, and the mother liquor poured into a test-tube and evaporated to 1 c.c. By rubbing the liquid against the side of the tube, crystallization sets in. One c.c. of water is then added and the liquid filtered through a plug of cotton-wool. A few drops of HCl are then added. If over 1 per cent of cresotic acid be present, it separates and melts when the water is boiled, sinking to the bottom of the tube as oily drops.

Cresotic acid may be determined, if previously found to be present, by titrating the sample with baryta water. But unless a comparatively large amount be present, the results are not reliable, for the following reason. One grm. of salicylic acid requires 726.3 c.c. of centinormal baryta water for neutralization, whereas 1 grm. of cresotic acid only requires 659.4 c.c. Each 1 per cent of cresotic acid as an impurity therefore only lowers the quantity required by 0.67 c.c. It is impossible to work accurately on so enormous a volume, so that by using 0.2 grm. of the sample, this difference is reduced to 0.134 c.c., which means that an error in titration of $\frac{1}{10}$ of a c.c., where over 100 c.c. of titration liquid are used, entirely vitiates the result. If it be necessary to use this process 0.2 grm. of the acid (previously purified by solution in ether, filtration, evaporation, and drying at 60°) are placed in a flask, a few drops of alcoholic solution of phenol-phthalein added, and the centinormal solution run in to near the neutral point. The flask is then shaken over a flame, and directly the remainder of the acid is dissolved, the titration is completed.

Ewell and Prescott (*v. "Analyst,"* XIII. 237) distil with lime and so convert the cresotic acid present into cresol, 15 grm. of the acid and 15

gram. of CaO are dried and well mixed together with 15 gram. iron filings. The mass is distilled in a retort and the distillate collected. It is treated with just enough water to liquefy it and the liquid mixed with an equal volume of 9 per cent aqueous NaOH. Water is then added until precipitation commences. From the volume of water necessary to cause precipitation the proportion of cresotic acid may be approximately calculated by the following table, but amounts under 5 per cent are not to be detected with certainty :—

Volume of H ₂ O necessary for Precipitation.	Per cent of Cresol in Distillate.	Per cent of Cresotic Acid in Sample.
6.7	5	4.9
6.0	10	9.8
5.25	15	14.8
4.5	20	19.8

The Detection of Salicylic Acid.—An acidified alcoholic solution of salicylic acid is slowly reduced by sodium if warmed. The reduction product is salicylic aldehyde, easily recognized by its characteristic odour of meadow-sweet. On heating salicylic acid with H₂SO₄ and methyl alcohol, methyl salicylate is formed which has the characteristic winter-green odour. Curtman ("Jour. Chem. Soc." LI. 188) recommends the latter test being carried out if salicylic acid be suspected in wine, etc., in the following manner. Four c.c. of the wine or beer should be mixed with methyl alcohol (CH₃OH) and then 2 c.c. of pure HSO₄ added cautiously. The liquid is agitated, heated for 2 minutes, allowed to cool and then heated to boiling. If salicylic acid be present, the odour of winter-green is perceptible. If only minute traces be present, it may be necessary to heat a third time.

Jorissen gives the following reaction, which is not yielded by benzoic or cinnamic acids. A solution of salicylic acid or a salicylate is treated with sodium nitrate and acetic acid and then a drop or two of copper sulphate solution, and the liquid boiled, when, if salicylic acid be present, a blood-red colour results.

The test, however, on which practically every analyst relies is the intense violet colour produced by solution of salicylic acid with a drop of neutral solution of ferric chloride. The test answers with solutions containing 1 in 110,000. The colour is destroyed by alkalis and most acids. In examining foods, wines, etc., suspected of containing salicylic acid it is necessary to remove various interfering substances, so that the liquid (or the extract by dilute KOH solution from the solid matter) is acidified and extracted with ether, petroleum ether or chloroform. The best solvent is a mixture of equal volumes of ether and petroleum ether. The solvent is extracted with water containing a trace of free alkali and the aqueous liquid exactly neutralized with HCl, and a few drops of ferric chloride solution (or better, solution of iron-alum) added, when a violet colour results if salicylic acid be

present. If minute quantities only are present, the ether may be evaporated and the residue tested with the iron solution.

Or the residue from the evaporation of the solvent may be treated with 2 drops of HNO_3 and the ammonia added. The acid is converted into picric acid, and a thread of white wool will be dyed a deep yellow if treated with the few drops of liquid.

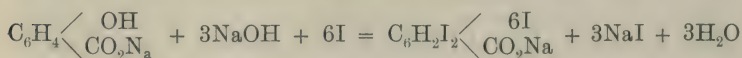
Vitali ("Répertoire de Pharmacie," [3], 19, 39) prefers toluene as the solvent as it does not dissolve out any interfering substances at all. He also confirms the presence of salicylic acid by adding a drop of dilute H_2SO_4 and 1 drop of a very dilute and almost colourless solution of copper sulphate to the residue left by evaporation of the solvent. In the presence of a minute trace of salicylic acid, a green spot will remain on evaporation.

S. Harvey ("Analyst," xxviii, 2) describes the quantitative application of this reaction to salicylic acid in wine, beer, etc., his method being a slight improvement on previously described similar processes. In the author's laboratory this has been found to give very accurate results, using a solvent consisting of equal volumes of ether and petroleum ether.

An aqueous 1 per cent solution of iron-alum, to which a few drops of H_2SO_4 have been added as a preservative, is recommended for the colorimetric determination of salicylic acid. The tint given by this reagent is more definite and persistent than that obtained with Fe_2Cl_6 . The acid is extracted from a known volume of the previously acidified solution by two successive shakings out with ether. The bulked ether extracts are then shaken out with $\frac{\text{N}}{2}$ or $\frac{\text{N}}{10}$ alkali, the alkaline solution exactly neutralized with acid and diluted to a definite volume of 250 c.c. or 500 c.c. One hundred c.c. of this solution are treated, in a Nessler glass, with 2 c.c. of the iron-alum reagent, and the colour matched with a known volume of freshly prepared standard solution of salicylic acid containing 0.001 gram. or 0.0001 gram. of salicylic in each c.c.

No larger quantities than 2 mgs. of salicylic acid should be used for the actual determination.

Messinger and Vortmann ("Berichte," xxii. 2312, and xxiii. 2753) have proposed an iodometric process based on the addition of an excess of standard iodine to the acid in a dilute alkaline solution. The acid is precipitated as a substituted iodo compound and the excess of iodine is determined by titration with thiosulphate solution. Six atoms of iodine enter into the reaction with one molecule of salicylic acid:—

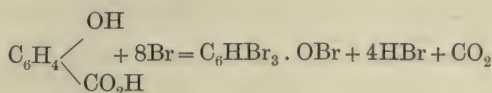


One c.c. of decinormal iodine corresponds to 0.0023 gram. of salicylic acid. In order to get the best results, about 0.15 gram. of salicylic acid is the best quantity to work upon; the solution should not contain more than 0.5 c.c. of a 10 per cent solution of NaOH : excess of decinormal iodine is added at about 50°C ., and the liquid, in a closed

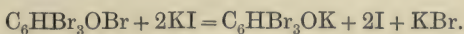
flask, allowed to stand for a few minutes. Excess of dilute sulphuric acid is then added, the liquid filtered, the precipitate washed with water and the filtrate titrated with standard thiosulphate solution.

Fresenius and Grunhüt ("Zeit. Anal. Chem." xxxviii. 292) have criticized this method very adversely, but as long as carried out carefully, avoiding excess of alkali, fairly good results may be obtained.

Freyer's process ("Chem. Zeit." xx. 820) is one of the best methods for the determination of salicylic acid, where the quantity is not too small (when of course the colorimetric process must be adopted). This process is based on the fact that bromine water reacts with salicylic acid as follows:—



Excess of bromine water is added to the salicylic solution, whereby the bromine compound is precipitated and the excess of bromine liberates iodine from potassium iodide; the tribromphenol bromide also enters into the reaction.



So that in calculating the results, only 6 atoms of bromine correspond to 1 molecule of salicylic acid. This process should be carried out as follows. A known weight of the sample is dissolved in water with the aid of a *little* caustic soda, and a measure corresponding to about 0.1 gm. of salicylic acid is diluted to about 100 c.c. with water, in a stoppered bottle. Ten c.c. of HCl are added, and then a known volume (50 to 60 c.c.) of a solution of sodium bromide and bromate (about 10 gm. of NaBr and 3 gm. of NaBrO₃ per litre) added. This solution is prepared by adding bromine to caustic soda solution, or by dissolving the salts themselves. The bottle is closely stoppered, well shaken and stood in the dark for an hour. A blank experiment is conducted at the same time omitting the sample only. Excess of 10 per cent solution of potassium iodide is then added and the liberated iodine titrated with decinormal thiosulphate solution. Each c.c. decinormal thiosulphate is equivalent to 0.0023 gm. of salicylic acid, so that the excess of thiosulphate required for the blank experiment allows the amount of salicylic acid to be calculated. Owing to the fact that starch must not be added till quite close to the end reaction, substances containing starch should be extracted with 90 per cent alcohol and the determination carried out on the extract. In substances containing sulphites or sulphurous acid, the liquid, such as wine, etc., must be rendered alkaline, concentrated, rendered acid and extracted with ether and petroleum ether. The solvent is then extracted with dilute alkali and this aqueous solution used for the determination. Salicylic acid may be estimated in this manner in the presence of benzoic acid, which does not react with the bromine solution.

For the examination of milk, fat and proteids should be removed

by adding a mercuric nitrate in acid solution, and filtering the liquid. The above methods can then be applied to the clear whey.

Metallic salicylates can be assayed direct if soluble in water, or by dissolving in acid and precipitating the metal with KOH if not, and examining the alkaline filtrate.

Seidell ("J. Amer. Chem. Soc." 1909, **31**, 1168) considers that both Freyer's and Messinger and Wortmann's process are of uncertain value and strongly recommends the precipitation of dibrom-salicylic acid as more reliable. This process, which is carried out as described below, has given excellent results in the author's hands.

About 0.25 gm. is placed in a stoppered flask and a few c.c. of water and about 50 c.c. of strong HCl added. $\frac{N}{5}$ bromine solution (in HCl) is added, until after well shaking and warming to about 90°, the yellow colour of the last two drops of bromine solution is persistent for five minutes.

The reading may then be taken as final, four atoms of bromine representing one molecule of salicylic acid. The results are, in the author's experience, within 2.5 per cent of the truth.

CAMPHOR.

Camphor is official in the Pharmacopœia, being described as a white crystalline substance obtained from *Cinnamomum camphora*.

Its specific gravity is given as about 0.995. It forms a liquid when triturated with menthol, phenol, or thymol. Its solubility in water is officially given as 1 in 700; in alcohol (90 per cent) 1 in 1; in chloroform 4 in 1, and in olive oil as 1 in 4.

Camphor $C_{10}H_{16}O$, is the stearoptene of the essential oil of *Laurus camphora* (*Cinnamomum camphora*). The commercial article is dextrorotatory, the much rarer lævorotatory variety occurring in the oil of *Matricaria parthenium*. Camphor has also been prepared synthetically, but in the optically inactive variety. Camphor forms a translucent, colourless mass (or powdery "flowers") melting at 175° and boiling at 204°. It is dextrorotatory, the specific rotation varying with the solvent. Landolt's formula gives the specific rotation for different degrees of concentration:—

$$[\alpha]_d = +55.4^\circ - (a \times q)$$

where q is the number of grms. of solvent in 100 grms. of solution, and a is a constant for each solvent. For alcohol $a = 0.1372$ and for benzol 0.1632. Thus the apparent specific rotation of camphor, when 10 grms. are dissolved in 90 grms. of alcohol is

$$+55.4^\circ - (90 \times 0.1372^\circ) = +43.052^\circ.$$

The most exhaustive determinations are those of Partheil and Van Haaren ("Journ. Soc. Chem. Ind." 1900, 684). They show that the more dilute the alcohol, the lower the apparent specific rotation. Also, as the percentage of camphor increases, the apparent specific rotation diminishes. They give the formula $P = 1.5152 a$, where P is the

percentage of camphor by weight, and α is the observed rotation for 200 mm.

To ascertain the volume percentage, the specific gravity of the alcohol must be arrived at. This is given by the formula :—

$$S = \frac{100 - p}{\frac{100}{a} - 1.05 p} \quad \text{where } S \text{ is the specific gravity of the alcohol, } p \text{ is the}$$

percentage by weight of camphor and a is the specific gravity of the alcoholic solution of camphor. They give the following table :—

No.	Specific Gravity of Alcohol.	Camphor.	Rotation for 200 mm.	Specific Rotation.
		Per cent		
1	0.7896	10	+ 6.98°	43.4362
2	0.8212	10	+ 6.78°	40.6666
3	0.8505	10	+ 6.69°	39.0439
4	0.8637	10	+ 6.65°	38.1439
5	0.8781	10	+ 6.60°	37.2755
6	0.8909	10	+ 6.59°	36.7622
7	0.9007	10	+ 6.59°	36.4008
8	0.7895	8.37	+ 5.79°	43.2142
9	0.7895	6.81	+ 4.69°	43.1411
10	0.9007	8.35	+ 5.48°	36.2929
11	0.9007	6.82	+ 4.40°	35.6951

The above formula enables the camphor to be accurately determined in spirit of camphor.

The Spirit of Camphor of the British Pharmacopœia is a solution of 1 ounce of camphor in 9 fluid ounces of 90 per cent alcohol (the volume of the camphor when in solution being almost identical with that when in the solid state).

It should have a specific gravity about 0.850, and should have an optical rotation in a 200 mm. tube of +7.7° to +7.8°, equivalent to about 11.7 per cent of camphor by weight. Schmatolla ("Apoth. Zeit." xvi. 290) proposes the following method for determining the camphor in spirit of camphor. Ten grms. are placed in a 50 c.c. burette, and shaken with 30 c.c. of saturated solution of salt. When the camphor has risen to the surface, exactly 1 c.c. of petroleum ether is poured in and the camphor dissolved by careful agitation. The volume of the petroleum layer is then read off. After subtracting the original 1 c.c. each c.c. increase in the volume corresponds to 0.99 gm. of camphor.

The specific rotation of camphor in olive oil is a matter of importance as it enables its rapid determination in *Linimentum camphoræ* (camphorated oil) to be made.

Linimentum camphoræ (camphorated oil) is a solution of 1 ounce of camphor in 4 fluid ounces of olive oil. It therefore contains 21.45 per cent by weight of camphor. The apparent specific rotation of camphor in olive oil varies from +52° to +55° according to concentration. Leonard and Smith ("Analyst," xxv. 202) give the following

figures, after correcting for the slight optical activity of olive oil, which varies from $+0.1^\circ$ to $+0.2^\circ$ for a 200 mm. tube:—

	A	B	C	D
Percentage of camphor by weight	5.32	11.26	20.66	26.78
Specific gravity at 15.5°	0.91903	0.92173	0.92173	0.92911
Observed rotation (200 mm.)	$+5.26^\circ$	$+11.35^\circ$	$+20.74^\circ$	$+26.79^\circ$
Angular rotation for 1 per cent camphor	0.964°	0.998°	0.998°	0.996°
Apparent specific rotation of camphor	$+52.4^\circ$	$+54^\circ$	$+53.9^\circ$	$+53.6^\circ$

It is clear, therefore, that each 1 per cent of camphor, when the amount is over 10 per cent, produces practically 1° of rotation. Hence a calculation is easy and the camphor can rapidly be determined.

Liversege ("Chemist and Druggist," LVIII. 167) gives the following formula for determining the amount of camphor per 100 grms. of sample:—

$$P = \frac{100 (L - o)}{(C - o) (S + kP)}$$

Where P is the percentage of camphor; L the rotation in a 200 mm. tube; C = 104 (that is twice the specific rotation of camphor in olive oil); o is the rotation of the oil itself, if any, in a 200 mm. tube (this may be taken as about $+0.2^\circ$); S is the specific gravity of olive oil, say 0.915; and k is the increase in specific gravity produced by 1 per cent of camphor, which is about 0.0004.

The camphor may also be determined gravimetrically by evaporation. Three to 5 grms. are heated in a flat-bottomed capsule at 120° for two or three hours. The loss, after adding 0.15 per cent to compensate for gain in weight of the oil, due to oxidation, may be taken to be camphor. The oil itself can be examined for mineral or nut oil, etc.

CHLOROFORM.

Chloroform CHCl_3 , is, when pure, a liquid of specific gravity 1.5020 at 15° , and boils at 60.8° . The British Pharmacopœia, however, requires it to contain a little absolute alcohol in order to hinder decomposition.

It is officially described as follows:—

Specific gravity 1.490 to 1.495. Boils between 60° and 62° . If 20 c.c. be allowed to evaporate from filter paper on a warm plate, no foreign odour is perceptible at any stage of the evaporation. Water shaken for five minutes with half its volume of chloroform is neutral to litmus, and gives no colour with 1 c.c. of a 5 per cent solution of cadmium iodide and a few drops of starch solution (absence of acid and of free chlorine), and should not yield more than a very slight

opalescence with four drops of silver nitrate solution (absence of chlorides). After shaking H_2SO_4 with 10 volumes of chloroform for twenty minutes, and setting aside for fifteen minutes, both the acid and the chloroform should be transparent and nearly colourless. Two c.c. of the H_2SO_4 layer, diluted with 5 c.c. of water, should remain transparent and almost colourless and should have a pleasant odour. On further dilution with 10 c.c. of water, and the whole stirred with a glass rod and four drops of silver nitrate solution added, the transparency should only be slightly diminished. Water which has been shaken with half its volume of chloroform, first treated as above with H_2SO_4 , should show a transparency which is only slightly diminished with silver nitrate solution. It should evaporate without residue.

The purity of chloroform is a matter of the highest importance on account of its use for anæsthetic purposes. Impurities may be present as a result of faulty manufacture, or as the result of decomposition.

The following are products of decomposition: chloro-carbonic ether $\text{C}_2\text{H}_5\text{CO}_2\text{Cl}$; carbon oxychloride COCl_2 ; possibly allylene dichloride $\text{C}_3\text{H}_4\text{Cl}_2$; hydrochloric, hypochlorous, and, possibly, formic acids.

Ethylene dichloride $\text{C}_2\text{H}_4\text{Cl}_2$ and ethyl chloride $\text{C}_2\text{H}_5\text{Cl}$ may be present as impurities.

The deliberate adulteration of chloroform is not common.

The proportion of alcohol in chloroform may be approximately deduced from the specific gravity. According to Schacht ("Pharm. Journ." [3], xxiii. 1005), the following figures are accurate:—

Pure chloroform	1.5020 at 15°
" "	with 0.25 per cent alcohol	1.4977
" "	" 0.5 " " "	1.4939
" "	" 1.0 " " "	1.4839
" "	" 2.0 " " "	1.4705

Oudemans ("Zeit. Anal. Chem." xi. 409) determines the alcohol, by estimating the solubility of pure cinchonine in the chloroform. If 10 c.c. be well shaken for an hour at 17° with excess of dry cinchonine, filtered, and 5 c.c. evaporated and the residue weighed, the following amounts will be obtained:—

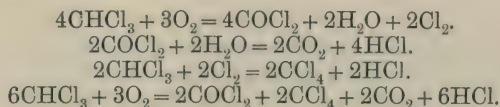
Pure chloroform021 grms.
" "	with 1 per cent alcohol067 "
" "	" 2 " " "111 "
" "	" 3 " " "152 "
" "	" 4 " " "190 "
" "	" 5 " " "226 "
" "	" 6 " " "260 "
" "	" 10 " " "346 "

Organic Impurities.—Brown prefers to detect impurities which have an offensive odour by a more extended test than the simple evaporation of the chloroform, as required by the Pharmacopœia. He states ("Pharm. Journ." [3], xxii. 769) that by careful fractional distillation, and dividing the sample under examination into two fractions, one of 10 per cent, the other of 75 per cent, and a residue of 15 per cent, both the more and less volatile impurities are obtained in

a concentrated form, and thus betray themselves by their odour. The non-volatile impurity may be determined by slow evaporation, with precautions to exclude dust, at a temperature of about 90° F. This process, which may be considered as an extension of the present test of the British Pharmacopœia, requires about 130 c.c. of the sample, and several days' time for each experiment. In order to show this, the following test, based on observations by Ramsay, who considered that traces of carbonyl chloride in a sample of chloroform examined, were responsible for the death of a patient, has been recommended as being the best for detecting incipient decomposition in chloroform. To 5 c.c. of the chloroform in a test-tube, 4 c.c. of perfectly clear, saturated solution of barium hydroxide are added without agitation, and the tube securely closed. It is then set aside in a dark place for six hours, when no film should be found at the contact of the two liquids.

Brown (who originally suggested the cadmium iodide test in the form of zinc iodide, however) at one time considered the baryta water test the more delicate, but as the result of exhaustive investigations ("Pharm. Journ." [3], xxiii. 792) he considers the iodide test the most reliable. He states that during the first stages of decomposition a distinct reaction is obtained with zinc iodide and starch, but none with baryta water, a separation of water being also observed. After further decomposition, zinc iodide and starch give a more marked reaction than at first, and baryta water also reacts, but faintly. Still following the decomposition, it is found that both reagents continue to give marked reactions until a point is reached, when that produced by zinc iodide and starch is observed to become less marked, and finally to disappear altogether, while the reaction with baryta water may still be obtained. A small quantity of deep straw-coloured liquid is also observed at this stage floating on the surface of the chloroform. At this point there remains a considerable quantity of undecomposed chloroform, which may, either before or after separating the decomposition products, be again put into an active state of decomposition by simply removing the stopper from the bottle for a few seconds, replacing it, and again exposing it to sunlight, when reactions similar to those already described with zinc iodide and starch are obtained. The author thinks that results such as those described could not have been obtained, if Professor Ramsay were correct in stating that carbonyl chloride and hydrochloric acid are the only products obtained from chloroform decomposing in the presence of air.

The following equations are given as a probable explanation of the changes observed :—



In harmony with this view, chlorine, water, and carbonyl chloride are found in the early stages, the chlorine being first recognized, and disappearing with the water at a more advanced stage, and the carbonyl

chloride reaction being invariably obtained, not only in the early but also in the most advanced stage met with.

The German Pharmacopœia requires chloroform used for anæsthesia to remain colourless for forty-eight hours when shaken with H_2SO_4 ; and also that if 20 c.c. be shaken with 15 c.c. of H_2SO_4 and 4 drops of formaldehyde solution in a glass stoppered flask previously rinsed out with sulphuric acid, the acid should remain colourless for half an hour.

The presence of aldehyde or acetone is indicated by boiling the sample with aqueous potash, which is darkened if aldehyde or acetone be present.

Chloroform made from pure ethyl alcohol is naturally easier to purify than when made from methylated spirit or acetone. But by very careful purification it can be made from the two last-named sources to satisfy the requirements of the British Pharmacopœia, which now includes chloroform from any source so long as it is sufficiently pure. For purposes of comparison pure chloroform may be made from chloral hydrate, or by crystallization with salicylide (Anschutz's process).

Chloral Hydrate.—This body is a combination of trichloraldehyde with water to form the crystalline trichlorethylidene glycol $\text{CCl}_3 \cdot \text{CH}(\text{OH})_2$.

The Pharmacopœia requires it to be soluble in less than its weight of water, alcohol, or ether, and in four times its weight of chloroform. The aqueous solution is neutral or faintly acid to litmus. When gently melted it commences to solidify at 48.9° . It boils at 94.4° to 96.7° . It leaves no residue on heating. If 4 grms. be heated with 30 c.c. of normal soda solution, not more than 6 c.c. of normal H_2SO_4 should be required to neutralize the alkali remaining after the reaction. A solution in chloroform when shaken with H_2SO_4 imparts no colour to the acid. If 1 grm. be warmed with 6 c.c. of water and 0.5 c.c. of 10 per cent KOH, the mixture filtered and iodine solution added until the liquid is of a deep brown colour and the whole set aside for an hour, no deposit of iodoform should take place (absence of chloral alcoholate). Its aqueous solution should yield no precipitate with silver nitrate solution (absence of free chlorides).

The official tests for chloral hydrate are not satisfactory. Many good samples do not commence to solidify after melting until a much lower temperature than that given, the United States Pharmacopœia allowing a wide range of temperature. At the same time a low solidifying point indicates excess of water, and a temperature of 47° should be insisted on. The quantitative test should be carried out in the cold, when accurate results are obtained, which is not the case when the mixture is heated. Each c.c. of normal alkali used is equivalent to 0.1475 chloral, or 0.1665 gr. of chloral hydrate.

A useful method of examining chloral hydrate is that proposed by K. Müller. Twenty-five grms. are placed in a finely graduated tube and a slight excess of strong caustic potash solution added. The tube must be kept cool. After remaining for two hours, the liquid becomes clear and separates into two layers, the lower layer being chloroform. The temperature is adjusted to 17° . The number of c.c. multiplied by

1.84 gives the weight of anhydrous chloral in the sample used, or by 2.064 the weight of chloral hydrate. Carefully carried out, this process yields results which are accurate to within 0.5 per cent.

Frequently a slightly higher yield of chloral than the theoretical (89.1 per cent chloral) is obtained, owing to the fact that this drug is often made not quite fully hydrated, in order to avoid deliquescence, or even, in hot weather, liquefaction.

TABLE OF CHEMICALS.

(Official requirements are in italics; non-official requirements are in ordinary type.)

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million. permittible.	As ₂ O ₃ per million. permittible.	Probable Adulterants.	Principal Tests.
—								
Acetanilide	115.5°	—	1 in 200	1 in 4	none	none	mineral matter	Should leave no ash. Treated with potash solution and a few drops of CHCl ₃ added, penetrating odour of phenyl isocyanate is given off. The official tests with ferric chloride are erroneous and meaningless.
Acidum aceticum	—	—	in all proportions	in all proportions	none	none	emphyreumatic matter	Specific gravity 1.044. 1 gm. requires 5.5 c.c. of normal alkali (= 33 per cent acetic acid). 2 c.c. diluted with 10 c.c. of water and 1 "drop" of 1 per cent solution of potassium permanganate should remain crimson for at least half a minute.
Acid. acetic, dilute	—	—	in all proportions	in all proportions	none	none	emphyreumatic matter	Specific gravity 1.006. 10 grms. require 7.1 c.c. of normal alkali (= 4.27 per cent of acetic acid). Same test for emphyreumatic matter as for acetic acid.
Acid. acetic, glacial	15.5°	117° to 118°	in all proportions	in all proportions	none	none	emphyreumatic matter	Specific gravity 1.058 (1.049 at 25°). 1 gm. requires 16.6 c.c. of normal alkali (= 99 per cent acetic acid). Same test for emphyreumatic matter as for acetic acid. Official melting-point is incorrect, and should be 14.8°.

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
Acid, arsenious	—	—	1 in 100	—	5	—	improperly purified	0.25 gm., dissolved quickly in water with 1.25 grms. of NaHCO ₃ should when well shaken with 3 successively added drops of HCl, decolorize 50.8 to 50.9 c.c. of decinormal iodine solution (99.8 per cent As ₂ O ₃).
Acid, benzoic	121.4°	249°	1 in 400	1 in 3	20	none	—	Melting-point is for pure acid. Acid from gum benzoin may melt at 120°. Heated in closed vessel with CaCO ₃ mass dissolved in dilute HNO ₃ and AgNO ₃ added, only slightest cloudiness should result (absence of chlorobenzoic acid). When warmed with its own weight of potassium permanganate and 10 times its weight of dilute sulphuric acid, it should not develop odour of benzaldehyde (absence of cinnamic acid). 0.2 gm. in 10 c.c. of water does not decolorize 2 drops of 1 per cent potassium permanganate at once.
Acid, boric	—	—	1 in 30 1 in 25	1 in 30 1 in 27	10	4	—	When carefully heated to expel combined water it yields 56.4 per cent of anhydride.
Acid, carbolic	not below 38.8°	not above 182°	—	—	none	none	—	Specific gravity at melting-point, 1.060 to 1.066. Optically inactive. One part of phenol, liquefied by the addition of 10 per cent of water, added to 1 volume of glycerin, forms a clear liquid which is not rendered turbid with 3 volumes more of water (absence of cresol).

Acid, carbolic, liquid	—	—	—	—	—	—	Specific gravity 1.064 to 1.069. Boiling-point gradually rises to not over 182°.
Acid, chromic (anhydride)	192°	—	2 in 1	—	none	1	Absence of more than traces of sulphates, as shown by BaCl ₂ with an aqueous solution.
Acid, citric	—	—	4 in 3 10 in 6	1 in 1.5	5	1	1 gm. requires 14.3 c.c. normal NaOH for neutralization. To contain no lead. One drop of solution of FeSO ₄ and a few drops of H ₂ O ₂ and the excess of KOH solution should not give a purple or violet colour with an aqueous solution of the acid. Not more than 0.05 per cent of ash. One gm. of the acid with 10 c.c. of H ₂ SO ₄ (specific gravity, 1.83 at least), kept at a temperature of 90° for one hour gives only a faint lemon-yellow, but no brown colour (absence of tartaric acid).
Acid, gallic	—	—	1 in 100	1 in 5 1 in 8	none	none	Loses 9.5 per cent on drying at 100°. Is not precipitated by solution of isinglass, albumen, alkaloids, or tartrated antimony (absence of tannic acid). No ash. [Gallic acid is precipitated by tartrated antimony, this test being incorrect.]
Acid, hydrobromic, dil.	—	—	in all proportions	in all proportions	4	2	Specific gravity = 1.077. 4 grms. should require 5 c.c. of normal alkali (= 10 per cent HBr). No solid residue.
Acid, hydrochloric	—	—	in all proportions	in all proportions	6	10	Specific gravity, 1.160. 1 gm. requires 8.7 c.c. normal alkali (= 31.79 per cent HCl). 0.1 gm. requires 8.7 c.c. $\frac{N}{10}$ silver nitrate solution for complete precipitation.
Acid, hydrochloric, dil.	—	—	in all proportions	in all proportions	2	4	Specific gravity 1.052. 1 gm. requires 2.9 c.c. of normal alkali (= 10.58 per cent HCl).

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—	Specific gravity, 0.997. 1 gm., rendered alkaline with normal alkali, and maintained faintly alkaline all the time, should require 8.7 c.c. of $\frac{N}{10}$ stov. r nitrate before a permanent precipitate forms (= 2 per cent HCN).
Acid, hydrocyanic, dil.	—	—	in all proportions	in all proportions	none	none	—	Carefully poured on to H ₂ SO ₄ , little or no darkening in colour should take place. No precipitate with solution of lead sub-acetate (absence of malic and sulphuric acids). Specific gravity, 1.210. Not more than 0.5 per cent solid residue. 1 gm. requires 8.3 c.c. normal alkali (= 75 per cent lactic acid). Should give no precipitate with copper sulphate (absence of sarcosolactic acid); and none, or slightest traces only on boiling with Fehling's solution. Neutralized with ZnCO ₃ , dried and extracted with alcohol, should yield no glycerin to the alcohol.
Acid, lactic	—	—	in all proportions	in all proportions	none	none	sugars glycerine	
Acid, nitric	—	121°	in all proportions	—	.25	5	—	Specific gravity 1.420. 1 gm. requires 11.1 c.c. of normal alkali (= 70 per cent HNO ₃). Should be free from sulphates (it rarely is, in practice); should not yield more than 0.005 solid residue (it nearly always contains more solid matter than this).
Acid, nitric, dil.	—	—	in all proportions	—	10	1	—	Specific gravity 1.101. 1 gm. requires 2.7 c.c. of normal alkali (= 17.44 per cent HNO ₃).

Acid, nitro-hydrochlorum	—	in all proportions	—	5	2	—	Specific gravity 1.070. 4 grms. require about 10 c.c. of normal alkali.
Acid, oleic	13.3° to 15.5°	insoluble	readily soluble	none	none	stearic and palmitic acids	Specific gravity 0.890 to 0.910. 1 gm. dissolved in 15 to 20 c.c. of 90 per cent alcohol, and 2 drops of phenol-phthalatin solution and neutralized with a 25 per cent aqueous solution of NaOH and then rendered faintly acid with acetic acid, gives a liquid, 10 c.c. of which, mixed with 10 c.c. of ether and 1 c.c. of a 10 per cent solution of lead acetate, remains clear or only slightly turbid (absence of more than traces of stearic and palmitic acids).
Acid, phosphoric (conc.)	—	in all proportions	in all proportions	12	5	—	Specific gravity 1.500. Contains 66.3 per cent of H_3PO_4 . 1 gm. mixed with 2.5 grms. of PbO in fine powder should on evaporation and ignition yield 2.98 grms. of lead salt. A diluted solution gives no precipitate with albumen nor with tincture of ferric chloride (absence of meta- and pyrophosphoric acids). Does not throw down any silica when diluted with water and allowed to stand. Gives no precipitate with mercuric chloride solution (absence of phosphorous acid).
Acid, phosphoric, dilute	—	in all proportions	in all proportions	3	2	—	Specific gravity 1.080. 1 gm. evaporated with 0.5 gm. PbO yields 0.6 gm. of lead salts. Contains 13.8 per cent H_3PO_4 . Other tests same as for the strong acid.
Acid, sulphuric	—	in all proportions	—	25	5	—	Specific gravity 1.843. 1 gm. requires 20.1 c.c. of normal alkali (= 98 per cent of H_2SO_4). HCl with a little Na_2SO_3 poured carefully on to the surface of the acid should not cause a red coloration at the junction of the liquids (absence of selenium).
Acid, sulphuric, dilute	—	in all proportions	in all proportions	4	1	—	Specific gravity 1.094. 1 gm. requires 2.8 c.c. of normal alkali (= 13.65 per cent H_2SO_4).

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent. alcohol.	Lead per million. permittible.	Ag ₂ SO ₃ per million. permittible.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—	—
Acid, sulphurous	—	—	in all proportions	in all proportions	none	2	sulphuric acid	<i>Specific gravity 1.025. Only gives slight precipitate with BaCl₂. 1 gm. requires 15.7 c.c. of $\frac{N}{10}$ iodine solution (=5 per cent SO₂).</i>
Acid, tannic	—	—	2 in 1 1 in 1	10 in 6 1 in 1	none	none	gallic acid	<i>No appreciable ash. Its solution should not yield a pink colour with a 5 per cent solution of KCN (absence of gallic acid).</i>
Acid, tartaric	—	—	1 in less than 1	1 in less than 3	8	1	—	<i>Its aqueous solution is dextrorotatory (a solution of 30 grms. per 100 c.c. in water has a rotation of +4° in a 100 mm. tube). 1 gm. requires 13.3 c.c. of normal NaOH for neutralization. No lead. Ash not more than 0.05 per cent. (Good samples often yield 0.06 per cent of ash.)</i>
Aether	—	below 40.5°	1 in 10	in all proportions	none	none	alcohol	<i>Not more than 10 per cent should dissolve in water when equal volumes are shaken together (absence of excess of alcohol). Specific gravity 0.735. Dissolves without coloration when added drop by drop to H₂SO₄. No solid residue. Neutral to litmus.</i>
Aether, acetic	—	73.9° to 77.8°	1 in 10	in all proportions	none	none	organic impurities	<i>Specific gravity 0.900 to 0.905. Neutral to litmus. No coloration when mixed with equal volume of H₂SO₄.</i>

Aether purifi- catus	not below 3-5°	1 in 11 1 in 10	in all proportions	none	water, methyl alcohol H ₂ O ₂	Specific gravity 0.720 to 0.722. Soluble in equal volumes of CS ₂ , absence of excess of H ₂ O). No coloration with KOH solution (absence of aldehyde). Neutral to litmus. Shaken with half its volume of dilute solution of K ₂ Cr ₂ O ₇ , acidified with H ₂ SO ₄ , no blue colour (absence of H ₂ O ₂). Methyl ether indicated by low boiling-point.
Alum	—	1 in 11 1 in 10	insoluble	2 none	—	Only slight coloration with potassium ferrocyanide. (Absence of more than traces of iron.)
Ammonium benzoate	—	1 in 6	1 in 30 1 in 22	2.5 none	—	No ash. Its solution should not be acid to litmus until it has stood for some time.
Ammonium bromide	—	2 in 3	1 in 15	none	chlorides.	0.5 gm. should require between 51.1 and 51.8 c.c. of $\frac{N}{10}$ silver nitrate solution (absence of excess of chloride).
Ammonium carbonate	—	1 in 4	1 in 200	5 none	—	1 gm. requires at least 18.7 c.c. of normal H ₂ SO ₄ (= 31.7 per cent NH ₃). This standard is too high, and commercial samples usually fall below it.
Ammonium chloride	—	1 in 3	1 in 60 1 in 55	6 2	—	No residue on ignition. No red colour with Fe ₂ Cl ₃ solution (absence of thiocyanate).
Ammonium phosphate	—	1 in 4 1 in 2	insoluble	10 4	—	Should contain 99.5 per cent of (NH ₄) ₂ HPO ₄ when determined by magnesium-ammonium sulphate. (2 grms. should yield 1.68 grms. of pyrophosphate.)
Amyl nitrite	—	almost in- soluble	in all proportions	none	—	Specific gravity 0.870 to 0.880. 70 per cent to distil between 96° and 100°. A mixture of 5 vols. with 95 vols. of alcohol should yield six times its volume of nitric oxide gas when tested as described under sp. ether nitros. (p. 485). Only a pale yellow colour with solution of KOH, (limit of aldehyde).

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million. permissible.	As ₂ O ₃ per million. permissible.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—	—
Antimony oxide	—	—	insoluble	insoluble	—	0.1 per cent	—	0.5 gm. dissolved in a solution of 1 gm. of cream of tartar and then mixed with 3 grms. of NaHCO ₃ requires 70 c.c. of N 10 iodine before a permanent colour appears (practically 100 per cent Sb ₂ O ₃).
Antimonious sulphide	—	—	insoluble	insoluble	—	0.1 per cent	—	Always contains arsenic.
Antimonium sulphuratum	—	—	insoluble	insoluble	—	0.1 per cent	—	3 grms., moistened and warmed with HNO ₃ until no red fumes are evolved and then ignited, yield 1.7 to 1.8 grms. of oxide (official figure is 2 gm., but is rarely obtained). If 1 gm. be dissolved in HCl and the liquid poured into a large excess of water, the precipitate washed and dried should weigh about 0.85 gm.
Antimonium tartaratum	—	—	1 in 17 1 in 15	almost insoluble	—	0.05 per cent	—	1 gm. dissolved in water with 2 to 3 grms. NaHCO ₃ requires 60.2 to 60.7 c.c. N 10 iodine, quickly added, to produce a permanent colour (= 99.2 to 100 per cent of tartrate). Note that the Pharmacopoeia gives two different solubilities in water !

Aqua. destillata	—	100°	—	in all proportions	none	none	solid residue organic matter ammonia	25 c.c. evaporated leave a scarcely visible residue. No reaction for metals, chlorides, nitrates, nitrites or sulphates. Neutral to litmus. 100 c.c. boiled for three minutes with 1 c.c. of dilute H ₂ SO ₄ and 0.1 c.c. of a 1 per cent solution of K ₂ Mn ₂ O ₈ should retain colour for an hour; with Nessler's reagent should not show more than 0.005 part of NH ₃ per million.
Argentī nitras	—	—	2 in 1	1 in 18	none	none	—	1 gm. should yield 0.843 gm. of AgCl when precipitated by HCl, and the resulting chloride washed and dried (100 per cent AgNO ₃).
Argentī nitras induratus	—	—	—	—	none	none	—	1 gm. yields 0.8 gm. of AgCl, similarly tested (= 95 per cent AgNO ₃).
Argentī nitras mitigatus	—	—	—	—	none	none	—	3 grms. yield 0.843 gm. of AgCl, similarly tested (= 33.3 per cent AgNO ₃).
Argentī oxidum	—	—	—	—	none	none	—	1 gm. yields 1.237 grms. AgCl, similarly tested (= 99.6 per cent Ag ₂ O).
Argentī iodidum	—	—	1 in 11	1 in 42	none	—	—	Should volatilize entirely on heating. The official statement that its aqueous solution is neutral is incorrect, as it at once decomposes and becomes acid.
Benzol	—	—	insoluble	—	none	none	—	Specific gravity 0.880 to 0.888. Commences to distil at 80° and about 90 per cent should distil below 100°, and the whole below 120°. Contains about 70 per cent of benzene and 20 to 30 per cent of toluene. Pure benzol C ₆ H ₆ boils entirely at 80° to 81°.
Bismuthi carbonas	—	—	insoluble	insoluble	—	4	—	1 gm. yields 0.99 gm. of bismuth sulphide when precipitated from dilute HCl solution (= 99.9 per cent oxycarbonate) yields at least 89.8 per cent Bi ₂ O ₃ on ignition.

	M. Pt.	B. Pt.	Solubility in water at ordinary °.	Solubility in 90 per cent alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
Bismuthi oxidum	—	—	insoluble	insoluble	—	4	—	1 grm. yields 1.1 grms. of bismuth sulphide (= 99.7 per cent oxide of bismuth). Loss on weight on ignition scarcely perceptible.
Bismuthi salicylas	—	—	—	insoluble	—	2	—	1 grm. yields 0.7 grm. of bismuth sulphide (= 98.6 per cent salicylate). On ignition leaves 62.64 per cent of Bi ₂ O ₃ . Shaken with alcohol, the alcohol should not yield a violet colour with ferric chloride solution (absence of free salicylic acid). As alcohol dissociates this salt, no samples will pass this test. Freedom from free salicylic acid is ensured by extracting with CHCl ₃ and shaking the extract with dilute ferric chloride solution, when no purple colour results.
Bismuthi subnitras	—	—	insoluble	insoluble	—	4	—	1 grm. yields 0.84 grm. of bismuth sulphide (= 99.7 per cent oxynitrate).

F. A. Smith ("Analyst," xxvi. 73) has pointed out that the sulphide determination is liable to error, and prefers the direct ignition to Bi₂O₃. The official formula for Bismuthi Subnitrat is BiNO₃ · H₂O, which corresponds to 76.3 per cent of Bi₂O₃. According to the British Pharmaceutical Codex commercial samples more nearly correspond to the formula (BiNO₃)₂H₂O which corresponds to 78.6 per cent Bi₂O₃. J. B. Harrison ("Analyst," xxxv. 118) has examined a number of samples manufactured by English and foreign firms and finds the Bi₂O₃ to vary between 78.8 per cent and 80.5 per cent.

Borax — — — 1 in 25 insoluble 4 6 — 1 grm. requires 5.2 c.c. of normal H₂SO₄ for neutralization (= 98.6 per cent borax), using methyl-orange as indicator.

Butyl chloral hydrate	about 77.8°	—	1 in about 50 1 in 44	5 in 3	none	—	No evolution of chloroform on heating with alkalis (absence of chloral). Should not discolour when warmed gently with H_2SO_4 .
Calcii carbonas	—	—	insoluble	insoluble	5	—	Should contain 98 to 99 per cent of CaCO_3 .
Calc. chloridum	—	—	1 in 1	1 in 3	2	—	Should contain 90 per cent of anhydrous CaCl_2 .
Calcii hydraz	—	—	1 in 900	—	5	CaCO_3	On ignition should lose about 25 per cent of its weight of water. Should contain 97 per cent of Ca(OH)_2 and not more than 2 per cent of CaCO_3 .
Calcii hypophosphis	—	—	1 in 8	insoluble	2	phosphates phosphites	0.25 grm. boiled for 10 minutes with a solution of 0.6 grm. of potassium permanganate should, on filtration, yield an almost colourless solution. Practically no precipitate with solution of lead acetate. Should contain at least 97 per cent of hypophosphite, as determined by precipitating phosphates, etc., by lead acetate, and, after standing for 12 hours, filtering, removing excess of lead and calcium by H_2SO_4 and alcohol, oxidizing with HCl and KClO_3 and precipitating the resulting phosphoric acid with magnesia mixture in the usual manner.
Calcii phosphas	—	—	insoluble	insoluble	5	—	Should contain at least 95 per cent of phosphate, as determined by dissolving in dilute HCl , and precipitating with NH_3 , and drying the ppt. at 100° .
Calx	—	—	1 in 900	—	5	Magnesia	Should contain 97 per cent CaO .
Calx chlorinata	—	—	—	—	10	—	Should yield 33 per cent of chlorine when tested as follows : 0.5 grm. mixed with 1.5 grms. of KI in 200 c.c. of water, and acidified with 6 c.c. of HCl , requires at least 46.8 c.c. of decinormal thiosulphate of sodium solution to decolorize.
Calx sulphurata	—	—	—	insoluble	—	—	0.8 grm. in H_2O with 1.4 grms. of copper sulphate and a little HCl should, when heated to near 100° , precipitate all the copper as sulphide, the filtered liquid yielding no reaction with potassium ferrocyanide (= 50 per cent of CaS).

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million. As_2O_3 per million.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—
Carbo ligni	—	—	insoluble	insoluble	none	Excess of mineral matter	<i>Maximum ash = 7.5 per cent.</i>
Carbon bisulphide	—	46° to 47°	1 in 500	readily soluble	none	Other sulphur compounds	A fetid odour indicates excess of impurities. <i>Specific gravity 1.268 to 1.269. No solid residue. Does not blacken lead acetate solution when shaken with it.</i>
Cerri oxalas	—	—	insoluble	insoluble	none	—	<i>When ignited should lose 53 per cent of its weight. Is always a mixture of cerium, lanthanum and didymium oxalates, as officially recognized.</i>
Creta preparata	—	—	insoluble	insoluble	none	Magnesium carbonate	<i>Should be free from barium carbonate, as shown by its acetic acid solution giving no ppt. with potassium chromate. Good samples should contain not more than 0.5 per cent insoluble in acetic acid, and at least 96 to 97 per cent of CaCO_3.</i>
Cupri sulphas	—	—	1 in 35	insoluble	—	—	Should contain 98 to 99 per cent pure crystalline sulphate of copper.
Ferri arsenas	—	—	insoluble	insoluble	—	—	Contains about 10 per cent <i>ferrous</i> arsenate, with <i>ferric</i> arsenate, oxide of iron, and H_2O of crystallization. <i>1 gm. dissolved in dilute H_2SO_4 requires 6.7 c.c. of decinormal potassium bichromate to oxidize the ferrous salt (= 10 per cent anhydrous ferrous arsenate).</i>

Ferri carbonas saccharatus	—	insoluble	3	3	—	Ferrous hydroxy-carbonate, more or less oxidized, mixed with sugar. 1 grm. dissolved in warm phosphoric acid requires 29 c.c. of decinormal potassium bichromate solution for oxidation (= about 33 per cent of ferrous carbonate).
Ferri et ammonii citras	—	2 in 1 almost insoluble	—	2	tartrates	On ignition leaves 31 to 32 per cent of Fe_2O_3 . Heated with solution of caustic potash and filtered from Fe_2O_3 , no separation of crystalline potassium acid tartrate should result on adding excess of acetic acid neutral to litmus.
Ferri et quininiæ citræ	—	2 in 1 almost insoluble	—	2	tartrates	Should answer the test for tartrates as for ferri-et ammonii citras. Should contain 15 per cent of anhydrous quinine, as determined by rendering its aqueous solution alkaline with ammonia, extracting the alkaline liquid with ether and evaporating the solvent.
Ferri phosphas	—	insoluble	—	3	—	Is a mixture of ferrous and ferric phosphates with some iron oxides. 1 grm. in dilute HCl requires 28.2 c.c. of decinormal potassium bichromate for oxidation (= about 47 per cent $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$).
Ferri sulphas	—	1 in 1.5 insoluble	—	1	ferric sulphate	Should lose about 40 per cent of water at 100° . 1 grm. dissolved in water with a little H_2SO_4 requires 36 c.c. of decinormal potassium bichromate solution for oxidation.
Ferri suphas exsiccatus	—	insoluble	—	2	ferric sulphate	1 grm. should, when dissolved in water with a little H_2SO_4 , require 54.6 c.c. of decinormal solution of potassium bichromate to oxidize it (= 92.5 per cent of FeSO_4).
Ferrum	—	insoluble	—	200	—	Contains about 98 to 98.5 per cent of metallic iron.

	M. P.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	200	—	<p>If 0.25 gm. be added to a hot solution of 1 gm. of copper sulphate in 15 c.c. of water, the flask at once well corked and shaken for ten minutes, and the liquid rapidly filtered with the least possible exposure to the air, and acidulated with H₂SO₄, the liquid should require 33.7 c.c. decinormal potassium bichromate solution (= 75.5 per cent Fe): A lower standard for arsenic than 200 per million is impracticable. Copper may be present up to 1 in 5000.</p> <p>Yields 30 per cent of Fe₂O₃ on incineration, washing out the potassium carbonate with water. Results are 2 to 3 per cent too high.</p> <p>Specific gravity, 1.260. Does not reduce Fehling's solution. No darkening at ordinary temperature with an equal volume of ammonia and a few drops of silver nitrate solution. Not more than a faint coloration when shaken with an equal volume of H₂SO₄. No arsenic, as indicated by heating with water, zinc and HCl in a tube capped with mercuric chloride paper. No ash.</p> <p>Not more than a trace of non-volatile matter. Heated with excess of copper, yields 43.5 to 44 per cent of mercury. Should be entirely soluble in ether (absence of mercurous iodide).</p>
Ferrum redactum	—	—	insoluble	insoluble	—	—	—	
Ferrum tartaratum	—	—	1 in 1	slightly insoluble	—	—	—	
Glycerinum	—	—	in all proportions	in all proportions	none	3	—	
Hydargyri iodid. (rubrum)	—	—	almost insoluble	1 in 300	—	—	—	

Hydrargyri oleas	—	—	insoluble	—	—	—	Should yield at least 22 per cent of mercury.
Hydrargyri oxidum	—	—	insoluble	—	—	—	Should yield 92 to 92.5 per cent of metallic mercury.
Hydrargyri perchloridum	—	—	1 in 16 1 in 19	—	1 in 3 1 in 5	—	Should yield 72.8 to 73.8 per cent of metallic mercury.
Hydrargyri subchloridum	—	—	insoluble	—	insoluble	—	Should yield 84.4 to 84.9 per cent of metallic mercury. Ether extracts nothing from it (absence of HgCl_2). Warmed with KOH solution it turns black, but does not evolve ammonia (absence of ammoniated mercury).
Hydrargyrum	—	357°	insoluble	—	insoluble	—	Traces only of fixed residue. Specific gravity, 13.50.
Hydrargyrum ammoniatum	—	—	insoluble	—	insoluble	—	Yields, when heated with lime, 78 to 79 per cent of metallic mercury. Traces only of fixed residue.
Hydrargyrum eum creta	—	—	insoluble	—	insoluble	—	The HCl extract yields no precipitate with stannous chloride solution (absence of mercuric salts). Should yield 66.6 per cent soluble in HCl, of which at least 96 per cent should be CaCO_3 , as determined by precipitation as oxalate in the presence of ammonia.
Iodoform	115°	—	almost insoluble	none	1 in 80 1 in 120	picric acid	No fixed residue. Water extracts no colouring matter from it. Specific gravity 2.0. Should contain 96.5 to 96.7 per cent of iodine.
Iodum	about 115°	about 180°	1 in 5000 1 in 7000	none	1 in 12	moisture; iodine compounds	Entirely volatile. First portion of sublimate not to contain slender colourless crystals (absence of ICN). Soluble in CHCl_3 without turbidity (absence of H_2O). 1 gm. requires at least 78.4 c.c. of decinormal sodium thiosulphate solution for decolorization (= 99 per cent I). Specific gravity, 4.955.

	M. Pt.	B. Pt.	Soluble in water at ordinary t°.	Soluble in 90 per cent alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
Liquor ammoniæ	—	—	in all proportions	in all proportions	—	—	—	<i>Specific gravity 0.959. 1 gm. requires 5.9 c.c. normal H₂SO₄ (= 10 per cent NH₃).</i>
Liquor ammoniæ fortis	—	—	in all proportions	in all proportions	—	—	tarry matters	<i>Specific gravity 0.891. 1 gm. requires 19.1 c.c. normal H₂SO₄ (= 32.5 per cent NH₃). On addition of excess of HCl, no colour or tarry odour is developed.</i>
Liq. ammoniæ acetatis	—	—	in all proportions	in all proportions	—	—	—	<i>Neutral or only faintly acid. Rendered alkaline with excess of KOH and carefully distilled into a known volume of normal H₂SO₄, should yield 1.5 per cent of NH₃.</i>
Liq. ammoniæ citratis	—	—	in all proportions	—	—	—	—	<i>Neutral or only faintly acid. Rendered alkaline, and distilled carefully, should yield 2.6 per cent of NH₃.</i>
Liquor arsenicalis	—	—	in all proportions	in all proportions	—	—	—	<i>25 c.c. neutralized by HCl, and kept slightly alkaline with NaHCO₃, should decolorize 50.8 to 50.9 c.c. of decinormal iodine solution (1 per cent of As₂O₃).</i>
Liquor arsenii hydrochlor.	—	—	in all proportions	in all proportions	—	—	—	<i>25 c.c. (see Liquor arsenicalis) decolorizes 50.8 to 50.9 c.c. of decinormal iodine solution (= 1 per cent As₂O₃).</i>
Liq. bismuthi et ammon. citratis	—	—	in all proportions	—	—	—	deficiency in bismuth	<i>Specific gravity 1.070. 10 c.c. in 40 c.c. of water yield a precipitate with H₂S, which, when washed and dried, weighs 0.55 gm.</i>

Liquor calcei	—	—	in all proportions	—	—	deficiency in lime	24 c.c. should require 10 c.c. decinormal H_2SO_4 (minimum of 0.1 per cent CaO). Free from chlorides.
Liquor calceis chlorinatæ	—	—	in all proportions	—	—	—	Specific gravity about 1.055. 1 grm., with 0.5 grm. of KI in water and 1 c.c. of HCl, liberates iodine which requires 5.6 c.c. of decinormal solution of sodium thiosulphate to decolorize (2 per cent of available chlorine).
Liquor calceis saccharatus	—	—	in all proportions	—	—	—	Specific gravity 1.055. 10 grms. require 6.3 c.c. normal H_2SO_4 (= about 1.8 per cent CaO).
Liq. ferri acetatis	—	—	in all proportions	—	—	—	Specific gravity 1.031. 10 c.c. on precipitation with NH_3 yield 0.26 grm. of Fe_2O_3 .
Liq. ferri perchloridi	—	—	in all proportions	—	—	—	Specific gravity 1.110. 10 c.c. on precipitation with NH_3 yield 0.8 grm. Fe_2O_3 .
Liq. ferri perchloridi fortis	—	—	in all proportions	—	—	—	Specific gravity 1.420. 5 c.c. on dilution and precipitation with NH_3 yield 1.6 grms. of Fe_2O_3 .
Liq. ferri pernitritis	—	—	in all proportions	—	—	—	Specific gravity 1.107. 5 c.c. on precipitation with NH_3 yield 0.23 grm. Fe_2O_3 .
Liq. ferri persulphatis	—	—	in all proportions	—	—	—	Specific gravity 1.441. 5 c.c. on precipitation with NH_3 yield 1.04 grms. Fe_2O_3 .
Liq. hydrargyri nitratiss acidus	—	—	in all proportions	—	—	—	Specific gravity about 2.000. Should yield no black precipitate with alkalis (absence of mercurous salts). Should contain at least 30 per cent of mercury, which is determined as mercuric sulphide, the precipitate being washed with alcohol and CS_2 before being dried and weighed.
Liq. hydrargyri perchloridi	—	—	in all proportions	—	—	—	Specific gravity about 1.001. 100 c.c. should yield 0.132 grm. of HgS.

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent. alcohol.	Lead per million. AS ₂ O ₃ permissible.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—
Liq. hydrogenii peroxid	—	—	in all proportions	in all proportions	—	barium salts	<i>A few drops diluted with 10 c.c. of water containing a trace of potassium chromate and 10 drops of dilute H₂SO₄ and then 2 to 3 c.c. of ether, yield a blue layer between the liquids, and on shaking, the ether becomes blue. 1 volume treated in a brine-charged nitrometer with 10 volumes of a mixture of 1 volume of H₂O₂, 2 volumes of 5 per cent solution of potassium permanganate and 7 volumes of water, gives from 18 to 22 volumes of oxygen indicating 9 to 11 volumes of available oxygen in the solution. Residue not more than 0.5 per cent. No reaction for Barium.</i>
Liquor iodi fortis	—	—	in all proportions	in all proportions	—	—	Should contain 11.6 per cent by weight of iodine.
Liq. magnesii carbonatis	—	—	in all proportions	—	under 1	—	<i>No characteristic reaction for sulphates. On evaporating and calcining the residue, this should be from 0.8 to 0.95 per cent, which should be oxide of magnesium.</i>
Liq. plumbi subacetatis dilutus	—	—	in all proportions	—	—	—	Specific gravity 1.001. Alcohol 1.25 per cent by volume. Should contain 0.37 gm. of Pb per 100 c.c., which may be estimated as sulphide.
Liq. plumbi subacetatis fortis	—	—	in all proportions	—	—	—	<i>Specific gravity 1.275. 1 gm. requires 17 c.c. of decinormal H₂SO₄ for complete precipitation. Fresh solutions require 18 to 19 c.c.</i>

Liq. potasæ	—	—	in all proportions	—	—	Specific gravity 1·058. 9 c.c. require 10 c.c. of normal H_2SO_4 (= 6·19 grms. KOH in 100 c.c.). Should not effervesce on addition of acids.
Liq. potassii permanganatis	—	—	in all proportions	—	—	Should contain 1 per cent of potassium permanganate.
Liq. sodæ chlorinatæ	—	—	in all proportions	—	—	Specific gravity 1·054. 3·5 grms. with 1 grm. KI in 100 c.c. of water and 3 c.c. of HCl, liberate sufficient iodine to require at least 25 c.c. of decinormal solution of sodium thiosulphate (= about 2·5 per cent available chlorine).
Liq. sodii arsenatis	—	—	in all proportions	—	—	Specific gravity 1·001. 100 c.c. yield 1·02 grms. of magnesium ammonium arsenate, when precipitated with ammonia-magnesia mixture and the washed precipitate dried at 100° to 105°.
Liq. zinci chloridi	—	—	in all proportions	—	—	Specific gravity 1·530. Contains 26 per cent of zinc, which can be determined as sulphide.
Lithii carbonas	—	—	insoluble 1 in 70	10	2	1 grm. neutralized with H_2SO_4 and ignited leaves 1·479 grms. of sulphate (= 98·5 per cent Li_2CO_3). 1 grm. dissolved in 4 c.c. HCl, and evaporated to dryness, the residue should dissolve in 3 c.c. of absolute alcohol, and should not be rendered turbid by the addition of 3 c.c. of ether (freedom from other alkalis).
Lithii citras	—	—	1 in 2 almost insoluble	5	2	2 grms. dried at 100° lose 0·38 grm.; dried at 115·5° lose an additional 0·13 grm.; and ignited should leave 0·77 grm. of oxide (= 98·5 per cent nitrate). These figures are inaccurate and the drying should be done at 140°, when 0·51 grm. should be lost. 1 grm. should yield 0·57 grm. Li_2SO_4 , as described under lithii carbonas.

The best method for determining lithium in its salts is that of Mayer and Merling ("Zeit. Anal. Chem.," 1880, 562) as recommended by Bowden. It is as follows: To a solution of the salt about eight times its weight of pure crystallized sodium phosphate is added and sufficient caustic soda solution to render the whole decidedly alkaline, and the whole evaporated to dryness on a water bath. Sufficient water is then added to dissolve the soluble salts, and the whole gently heated and filtered after allowing to stand for twelve hours. The precipitate is washed with a mixture of equal volumes of ammonia and water, and the filtrate and first two washings evaporated to dryness and treated with water as before; any precipitate which falls is added to the bulk. After washing well with ammonia and water, the precipitate is dried at 100° till constant, and is then Li_3PO_4 .

	M. Pt.	B. Pt.	Soluble in water at ordinary t°.	Solubility in 90 per cent. alcohol.	Lead per million.	As_2O_3 per million.	Probable Adulterant.	Principal Tests.
—	—	—	—	—	—	—	—	—
Magnesia levis Magnesia ponderosa	—	—	insoluble	insoluble	18	5	magnesium carbonate	Does not effervesce with acids. 0.5 gm. requires 24.7 c.c. normal H_2SO_4 for neutralization (add excess of acid and titrate back with normal alkali).
Magnesiæ carbonas levis Magnesiæ carbonas ponderosa	—	—	insoluble	insoluble	18	5	—	5 grms. calcined should yield 2.1 grms. Should contain at least 98.5 per cent of magnesium carbonate as determined by titration with acid.
Magnesiæ sulphas	—	—	1 in 1 10 in 13	insoluble	—	—	—	0.5 gm. should yield 0.22 gm. of pyrophosphate when precipitated with ammonia-magnesia mixture and the washed precipitate ignited.
Menthol	42° to 43° 43° to 44°	217°	almost insoluble	5 in 1	—	—	—	Specific gravity 0.890 at 20°. Specific rotation about - 59° in alcohol. Should contain 99 per cent menthol as determined by acetylation (see under peppermint oil).

Naphthol (β -naphthol)	122°	286°	1 in 1000	1 in 2	none	none	α -naphthol	
Paraldehyde	—	124°	1 in 10 1 in 8.5	in all proportions	none	none	aldehyde	Specific gravity 1.217. 0.1 gm. in 10 c.c. of boiling water gives no violet precipitate with 10 drops of 3 per cent solution of ferric chloride (absence of α -naphthol). No fixed residue. Specific gravity 0.998. No coloration when standing for 2 hours with solution of KOH (absence of aldehyde). Solidifies at -2° to 0°, if well stirred. Specific gravity often as low as 0.995. Rarely answers the official test for aldehyde, which is too stringent.
Phenacetin	135°	—	1 in 1400	1 in 20 1 in 21	none	none	acetanilide paraphenetidin	No fixed residue. A cold saturated aqueous solution does not become turbid on addition of bromine water (absence of acetanilide). 0.3 gm. with 1 c.c. alcohol should not assume a red colour with 3 c.c. of water, when boiled with a drop of iodine solution (absence of paraphenetidin). 0.5 gm. melted with 2.6 grms. chloral hydrate, and well shaken, should not become violet, red or blue. Such coloration indicates paraphenetidin. Should not yield phenyl-isothiocyanate when tested as described under acetanilide.
Phenazone	113°	—	1 in 1 4 in 5	1 in 1.3	none	none	acetanilide	0.1 gm. NaNO ₂ and 12 c.c. of a 1 per cent solution of phenazone with 1 c.c. of dilute H ₂ SO ₄ yield a deep green solution. Acetanilide lowers melting-point considerably. Should not yield phenyl-isothiocyanate when tested as described under acetanilide.
Phosphorus	43.3°	—	insoluble	1 in 350 (absolute alcohol)	none	—	—	1 gm. should dissolve slowly on boiling with 5 c.c. of HNO ₃ and 5 c.c. water, and the resulting solution should yield only the slightest reaction for sulphates. Specific gravity 1.770.
Picrotoxine	192.2°	—	1 in 330 1 in 334	1 in 13 1 in 13.5	none	non	—	Soluble in 10 parts of 10 per cent KOH solution, and then reduces Fehling's solution on boiling. No fixed residue. Its aqueous solution is not precipitated by HgCl ₂ or PtCl ₄ solution (absence of alkaloids). If pure melts at 199° to 200°.

	M. Pt.	B. Pt.	Solubility in water at ordinary °.	Solubility in 90 per cent. alcohol.	Lead per million.	As ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	—	—
Plumbi acetas	—	—	<i>1 in less than 3.</i> <i>1 in 2</i>	<i>1 in 30</i> <i>1 in 20</i>	—	—	—	<i>Solution only slightly acid. 1 grm. should require 53.1 c.c. of decinormal H₂SO₄ for complete precipitation. An aqueous solution should yield a colourless precipitate with potassium ferrocyanide (absence of copper and iron).</i>
Plumbi carbonas	—	—	<i>insoluble</i>	<i>insoluble</i>	—	—	—	Should be soluble to the extent of at least 98 per cent in 20 per cent HNO ₃ . Heated to redness in a porcelain dish, should leave 84 to 85 per cent of residue.
Plumbi iodidum	—	—	<i>1 in 2000</i>	—	—	—	—	<i>Should dissolve entirely in a 10 per cent solution of NH₄Cl, should contain at least 99 per cent pure PbI.</i>
Plumbi oxidum	—	—	<i>insoluble</i>	<i>insoluble</i>	—	—	—	<i>Completely soluble in acetic acid and in dilute HNO₃. Commercial samples contain up to 4 per cent insoluble matter, but should yield at least 96 per cent of PbO, when estimated as sulphide or sulphate.</i>
Potassa caustica	—	—	<i>2 in 1</i>	<i>1 in 2</i>	—	—	—	<i>1 grm. in H₂O should leave only a trace of insoluble matter and should require at least 16.1 c.c. of normal H₂SO₄ for neutralization (= 90.1 per cent KOH).</i>
Potassa sulphurata	—	—	—	—	—	—	—	<i>About 50 per cent should be soluble in 90 per cent alcohol.</i>

Potassii acetas	—	—	2 in 1	1 in 2	—	—	—	If ignited, 1 grm. should leave a residue of K_2CO_3 which requires 10·2 c.c. normal H_2SO_4 for neutralization (= 98 per cent of CH_3COOK).
Potassii bicarbonas	—	—	1 in 4 1 in 3·2	almost insoluble	5	1	—	1 grm. leaves 0·69 grm. on ignition, which requires 10 c.c. of normal H_2SO_4 for neutralization.
Potassii bichromas	—	—	1 in 10	insoluble	—	—	—	5·66 grms. of ferrous sulphate (crystalline) in water acidulated with H_2SO_4 requires 1 grm. of potassic bichromas to oxidize it, using potassium ferricyanide as indicator (= 99 per cent of bichromate).
Potassii bromidum	—	—	1 in 2	1 in 200	none	none	chloride of potassium	1 grm. requires 83·7 to 85·4 c.c. of decinormal $AgNO_3$ for complete precipitation. Solution of ferric chloride should not give a red colour with a cold solution (absence of thiocyanates).
Potassii carbonas	—	—	1 in 1 4 in 3	insoluble	5	2	—	1 grm. requires 11·9 c.c. normal H_2SO_4 for neutralization. 2 grms. on ignition should yield 1·66 to 1·70 grms. residue (absence of excess of H_2O). (About 84 per cent anhydrous K_2CO_3 .)
Potassii chloras	—	—	1 in 16	1 in 1700	8	2	—	Only slightest reaction for chlorides.
Potassii citras	—	—	1 in 0·6	—	4	1	—	1 grm. on ignition requires 9·7 c.c. of normal H_2SO_4 for neutralization. Commercial samples contain more H_2O than this allows, and 9·5 to 9·6 c.c. is a more correct value.
Potassii iodidum	—	—	1 in less than 1	1 in 12	none	none	potassii chloride	1 grm. requires 59·5 to 61·9 c.c. of decinormal $AgNO_3$ for complete precipitation (at least 98 per cent KI).
Potassii nitras	—	—	1 in 4	—	none	none	—	
Potassii permanganas	—	—	1 in 20 1 in 18	—	—	—	—	1 grm. in H_2O acidulated with H_2SO_4 requires 31·2 c.c. of normal oxalic acid solution for complete decolorization (98 per cent $K_2Mn_2O_8$).

	M. Pt.	B. Pt.	Soluble in water at ordinary t°.	Solubility in 90 per cent. alcohol.	Lead per million. AS_2O_3 permissible.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	—	—	Determined as BaSO_4 , should contain 99 per cent of potassium sulphate.
Potassii sulphas	—	—	1 in 10	insoluble	—	—	1 c.c. when ignited requires 8.4 c.c. of normal H_2SO_4 for neutralization (99 per cent of tartrate).
Potassii tartaras	—	—	1 in 1	—	5	—	1 c.c. of the dry salt requires 5.2 c.c. normal solution of NaOH for neutralization. Should contain at least 97.5 per cent of acid tartrate. Traces only of calcium to be present.
Potassii tartaras acidus	—	—	1 in 200	insoluble	5	—	
Salicin.	201°	—	1 in 28	1 in 60	none	acetanilide	Odour of meadow-sweet (salicylic aldehyde) when heated with H_2SO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$. No fixed residue. On contact with H_2SO_4 gives a red colour. On hydrolysis by means of HCl yields dextrose, which after neutralization of the liquid reduces Fehling's solution on boiling. An aqueous solution is not precipitated by tannic acid or potassium-mercuric iodide (absence of alkaloids). No acetanilide reaction.
Salol	42° to 43°	—	almost insoluble	1 in 10 1 in 12	none	—	Gives violet colour when a few drops of ferric chloride solution are added to its solution in alcohol. On melting with NaOH and adding HCl , salicylic acid is precipitated and phenol is set free. An aqueous extract should give no violet colour with ferric chloride. (Absence of salicylic acid.) The presence of very small quantities of moisture lowers the melting-point considerably, hence it should be placed in a desiccator for 24 hours before its melting-point is determined.

Santonin	170°	—	very slightly soluble	1 in 40	none	none	acetanilide	With warm alcoholic KOH it gives a violet colour. No fixed residue. No acetanilide reaction.
Soda tartarata	—	—	2 in 3	insoluble	8	2	—	1 gm. ignited leaves a residue requiring at least 7 c.c. of normal H_2SO_4 for neutralization (98 per cent sodium potassium tartrate).
Sodii arsenas	—	—	1 in 6	—	—	—	—	1 gm. with 1 gm. of glacial acetic acid in 50 c.c. of H_2O requires 2.03 grms. of lead acetate for complete precipitation. Should not lose weight at 148.9° C. (Absence of hydrated salt.) The lead acetate assay is quite useless. Should yield 99 per cent arsenate when determined by the ammonium-magnesia method.
Sodii benzoas	—	—	1 in less than 2	1 in 24	none	none	—	An aqueous solution yields a flesh-coloured precipitate with ferric chloride, but not a violet colour (absence of salicylic acid). 1 gm. ignited leaves a residue which requires 6.8 to 6.9 c.c. of normal H_2SO_4 for neutralization (98 per cent benzoate).
Sodii bicarbonas	—	—	1 in 11 1 in 12	insoluble	5	2	—	1 c.c. requires 11.8 to 11.9 c.c. normal H_2SO_4 for neutralization (= 98.3 per cent NaHCO_3). 1 gm. in 20 c.c. H_2O should not at once yield a red colour with phenol-phthalein more than be discharged by 0.2 c.c. normal HCl (limit of carbonate). Should only contain traces of chlorides, sulphates or ammonium.
Sodii bromidum	—	—	1 in less than 2	1 in 16	none	none	chlorides	1 gm. requires from 95.8 to 97.8 c.c. of N^{10} AgNO_3 solution (97.9 per cent to 100 per cent NaBr). No red colour by addition of ferric chloride to its aqueous solution (absence of thiocyanates).
Sodii carbonas	—	—	1 in 2 1 in 1.6	insoluble	8	2	—	On heating it loses 62.93 per cent of water. 1 gm. of crystallized salt requires 6.9 c.c. of normal H_2SO_4 for neutralization. Only traces of chlorides, sulphates and ammonium.

	M. Pt.	B. Pt.	Solubility in water at ordinary t°.	Solubility in 90 per cent. alcohol.	Lead per million. permittible.	As ₂ O ₃ per million. permittible.	Probable Adulterants.	Principal Tests.
—	—	—	—	—	16	4	—	Should correspond with the crystalline salt, after allowing for the loss of 63 per cent of H ₂ O.
Sodii carbonas exsiccatas	—	—	—	—	10	1	—	Should contain 98 to 99 per cent NaCl, as determined by titration with AgNO ₃ .
Sodii chloridum	—	—	1 in less than 3	—	—	—	—	<i>Its solution reduces CuSO₄ on warming, with formation of a red precipitate of cuprous hydride; 0.5 gm. boiled for 10 minutes with 25 c.c. of water and 1.15 gm. of potassium permanganate and filtered should give a nearly colourless solution. Its solution should only give faint precipitate with lead acetate (limit of phosphates and phosphites). Should contain at least 96 per cent of hypophosphites as determined by the method described under Calcii hypophosphite.</i>
Sodii hypophosphis	—	—	1 in 1	1 in 30 1 in 20	—	—	—	<i>Not more than 5 per cent of moisture. 1 gm. of the dried salt requires not less than 66.5 c.c. of N AgNO₃ for precipitation (= 98.9 per cent NaI).</i>
Sodii iodidum	—	—	1 in less than 1 1 in 0.55	1 in 3	none	—	—	<i>0.1 gm. in water, with 1 gm. of KI and 5 c.c. of dilute H₂SO₄, should yield in a brine-charged nitrometer not less than 32.5 c.c. of nitric oxide (= 98 per cent NaNO₂), and the gas should be almost completely absorbed by a strong solution of FeSO₄. Often contains a trace of lead and is allowed to give a trace of precipitate with dilute H₂SO₄.</i>
Sodii nitris	—	—	1 in 1.2	1 in 50	—	—	—	

Sodii phosphate	—	—	1 in 6	—	8	5	—	On ignition loses 62·84 per cent of its weight. Only the slightest traces of sulphates or chlorides. Contains 98 to 99 per cent of crystalline phosphate, as determined by ammonia-magnesia mixture.
Sodii sulphas	—	—	1 in less than 0·5 at 25°	insoluble	5	2	—	Loses 55·9 per cent on heating. Should contain 99·9 per cent of crystalline sulphate when determined as BaSO ₄ . (1 gm. should yield 0·725 gm. BaSO ₄ .)
Sodii sulphis	—	—	1 in 3	insoluble	—	—	—	1 gm. requires from 77·7 to 81·7 c.c. of $\frac{N}{10}$ iodine for oxidation (= 97·28 per cent sulphite).
Sodii sulphocarbolas	—	—	1 in 6	1 in 150	—	—	—	A dilute aqueous solution, does not give a yellowish-brown precipitate with uranium nitrate (absence of salicylate); should not at once give a precipitate with BaCl ₂ (absence of sulphates). Should lose 15·5 per cent H ₂ O at 105°. On ignition leaves 30·6 per cent of Na ₂ SO ₄ .
Sodium	95·6°	—	—	—	—	—	—	1 gm. cautiously treated with water requires at least 42·6 c.c. normal H ₂ SO ₄ for neutralization (97·4 per cent sodium).
Sulphonal	125·5°	300°	1 in 450 1 in 500	1 in 50	none	none	—	No fixed residue. No reaction for chlorides or sulphates.
Sulphur	about 115°	440°	insoluble	very slightly soluble	—	2	—	No fixed residue. Specific gravity 2·03. If sulphur be shaken with solution of ammonia, and filtered, the liquid leaves no residue on evaporation (absence of arsenic sulphide). This test is erroneous, as traces of ammonium sulphate may be formed, which would be interpreted as arsenic. Bird's test should be applied.
Sulphur iodide	—	—	insoluble	—	—	—	—	Boiled with water, the iodine is vaporized, leaving about 20 per cent of sulphur.

—	M. Pt.	B. Pt.	Solubility in water at 90 per cent. ordinary t°.	Solubility in alcohol.	Lead per million.	AS ₂ O ₃ per million.	Probable Adulterants.	Principal Tests.
Thymol	51°	233°	1 in 1500	8 in 3	—	—	—	<i>Completely volatile at 100°. Crystals sink in water (but large crystals often floating to the occlusion of air bubbles). Specific gravity = 1.028. Completely soluble in alkaline solutions.</i>
Zinci acetat	—	—	1 in 2.5	1 in 40	—	—	—	Contains 99 per cent acetate when determined as ZnO. At 100° it loses about 16.4 per cent of its weight.
Zinci carbonas	—	—	insoluble	insoluble	—	—	—	<i>Absence of other metals.</i> Composition is somewhat variable, but contains 55 to 57 per cent zinc, when determined as ZnO.
Zinci chloridum	—	—	5 in 2	1 in 1	—	—	—	<i>Absence of other metals.</i> Always contains moisture.
Zinci oxidum	—	—	insoluble	insoluble	0.02 per cent	10	—	<i>Entirely soluble in ammonia when warmed (absence of metallic zinc). Absence of other metals.</i> Should yield at least 98 per cent on ignition with a few drops of HNO ₃ .
Zinci sulphas	—	—	1 in less than 1 in 0.7	insoluble	—	—	—	<i>Absence of other metals.</i> Heated to 230° loses about 43 per cent of water of crystallization.
Zinci sulphocarbolas	—	—	1 in 2.5	1 in 2.5	—	—	—	Absence of other metals and sulphates. On ignition leaves 14.6 per cent of ZnO, using a few drops of HNO ₃ .
Zinci valerianas	—	—	1 in 120	1 in 60	—	—	—	<i>On ignition leaves 26 to 30 per cent ZnO, using a few drops of HNO₃. Distilled with dilute H₂SO₄ the distillate does not at once render solution of copper acetate turbid, but forms oily drops which gradually form a white crystalline deposit (absence of butyrate).</i>

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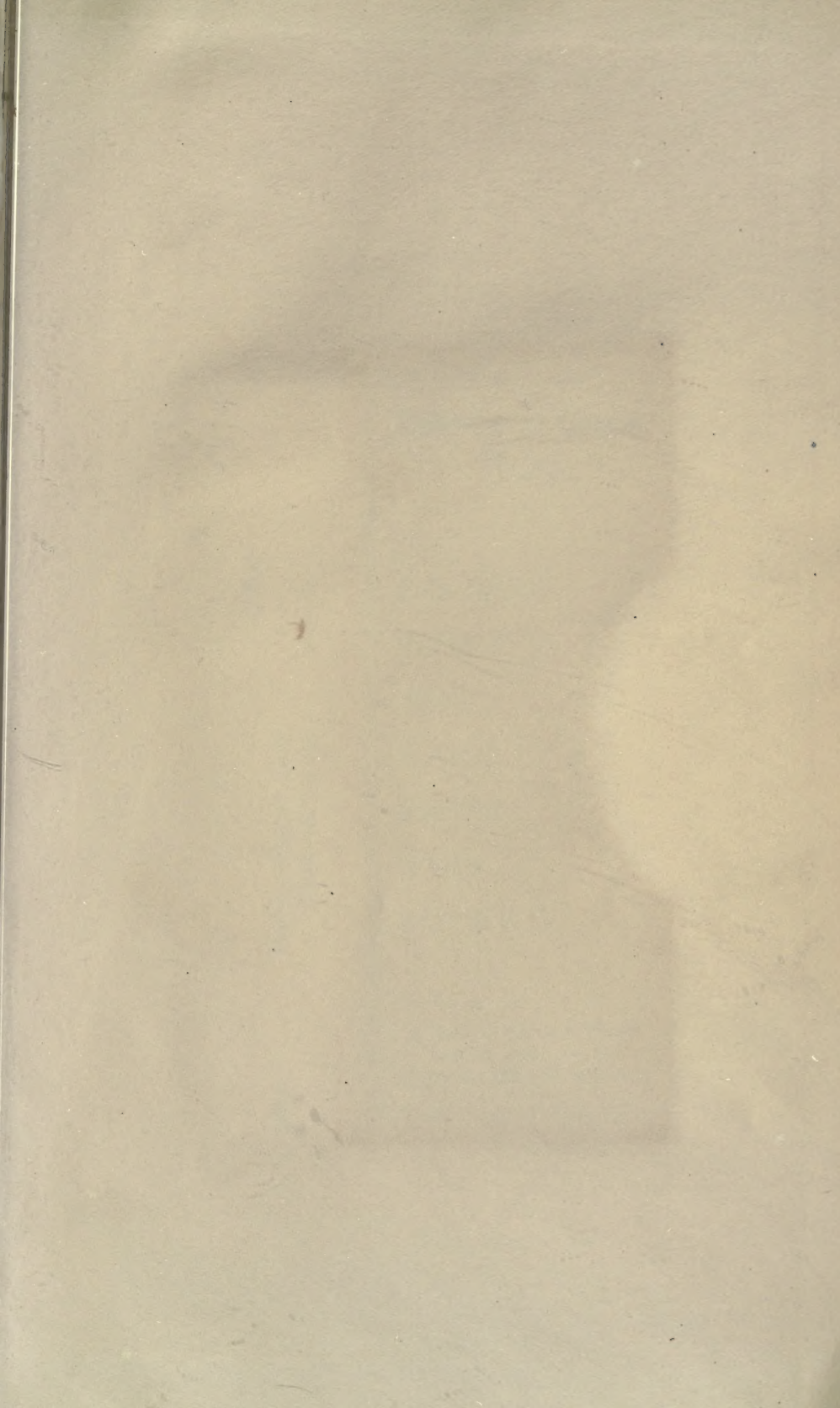
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